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(54) Title: A SAMPLE PRESENTATION APPARATUS FOR MASS SPECTROMETRY

(57) Abstract

A sample presentation device, with a surface-bound complex including at least one molecule which chemically modifies a biomolecule, is prepared and exposed to a biomolecule. The molecular weight of the chemically modified biomolecule is then determined by mass spectrometry.

Description

A Sample Presentation Apparatus For Mass Spectrometry

Background of the Invention

The present invention relates generally to derivatized mass spectrometry sample presentation apparatuses, and more specifically, to mass spectrometry sample presentation apparatuses
5   derivatized with complexes including at least one molecule which modifies a biomolecule.

Conventionally, mass spectrometry is a technique used to characterize analytes by determining their molecular weight. Ordinarily, mass spectrometry
10   involves the steps of: coating a sample presentation apparatus with an analyte, introducing the sample presentation apparatus into the mass spectrometer, volatilizing and ionizing the analyte, accelerating the ionized analyte toward a detector by exposing the ions
15   to an electric and/or a magnetic field, and analyzing the data to determine the mass to charge ratio of specific analyte ions.

If an analyte remains intact throughout this process, data will be obtained which corresponds to a
20   molecular weight for the entire intact analyte ion. Typically however, it is beneficial to additionally obtain data corresponding to the molecular weight of various fragments of the analyte. It is also beneficial to obtain data which only corresponds to the
25   pure analyte, even when impurities are present. Therefore, it is advantageous to be able to modify analytes by purifying and/or cleaving them, prior to determining their molecule weight.

In conventional mass spectrometry, the analyte is
30   either modified before it is coated on the sample

presentation apparatus, or during the volatilization
and ionization steps, which occur inside the mass
spectrometer. It is known that biomolecules (e.g.
polypeptides, deoxyribonucleic acid (DNA), ribonucleic
5   acid (RNA), or carbohydrates) can be selectively
digested at specific locations by exposing them to
immobilized complexes. However, it is disadvantageous
to modify an analyte before it is coated on the sample
presentation apparatus, as this extra step slows the
10   overall process, involves loss of the analyte, and can
possibly introduce contaminants. Moreover, if the
fragments are generated in solution in a reaction
between the analyte and a reagent, the kinetics of the
reaction may be rather slow, thus adding further delay.
15   It is also disadvantageous to generate fragments during
the volatilization and ionization steps as such methods
typically provide little control over the analyte
cleavage site and may lead to excessive degradation of
the analyte.
20   It is further known to use the Matrix-Assisted
Laser Desorption/Ionization ("MALDI") technique to
volatilize and ionize biomolecules in a mass
spectrometer. This technique involves surrounding a
biomolecule in a special matrix material. A LASER
25   beam, tuned to a frequency where the matrix absorbs, is
targeted on the matrix material. The LASER transfers
sufficient energy to volatilize a small portion of the
matrix material. A small number of analyte molecules
are thus carried along with the matrix material into
30   the vapor phase in the mass spectrometer.
         Prior to the development of MALDI, analysis of
biomolecules by mass spectrometry was quite difficult,
if not impossible, since no techniques were available
which were gentle enough to volatilize intact
35   biomolecules without any degradation or fragmentation.
While the MALDI technique provides an advantageous

technique for volatilizing biomolecules, this technique
does not provide for generation of analyte fragments or
purification of an analyte prior to introducing the
analyte onto a sample presentation apparatus. In
5 particular, the need for extra fragmentation or
purification steps slow the overall process, wastes
valuable analyte, and can introduce contaminants.

It is known from PCT Publication No. 94/28418 to
use a sample presentation apparatus for laser
10 desorption and ionization mass spectrometry, wherein
the sample presentation apparatus is derivatized with
surface associated molecules for promoting desorption
and ionization of biomolecules. In such surface-bound
biomolecule methods, the surface associated molecules
15 bind biomolecules, and later promote desorption of the
biomolecules when they are exposed to LASER radiation.
Biomolecule fragments are generated by exposing the
surface-bound biomolecules to reagents in solution, and
later washing away the reagent. This procedure is
20 problematic because it can introduce contaminants,
which reduces the quality of the collected data.
Specifically, if any reagent is left on the surface
after the washing procedure, it will be the first
material to be volatilized by the LASER beam, and may
25 very well swamp the signals from the material of
interest, thereby adversely affecting the data.

Moreover, such a process is difficult because it
relies on new and unproven systems for volatilizing
biomolecules. In contrast, MALDI matrix systems are
30 well studied, and known to effectively and gently
volatilize biomolecules. MALDI matrices cannot be used
with the surface-associated molecule method because the
matrix covers the surface-bound biomolecules, and thus
would completely prevent the acquisition of useful
35 data.

Furthermore, it is also disadvantageous to
directly bind biomolecules to a sample presentation
surface, because this would eliminate the possibility
of transferring a modified biomolecule to a second
5   region on the presentation surface or a second
presentation surface, where it could be exposed to
other modifying reagents. Therefore, any chemical
reactions according to the surface-bound analyte
method, must be performed sequentially, rather than in
10   parallel. In such sequential methods, the bound
analyte must be reacted with one reagent, introduced
into the mass spectrometer, removed, and then
subsequently reacted with a second reagent. Each of
these steps is disadvantageous as it presents an
15   opportunity for contaminating the sample and introduces
further delay.
        For the foregoing reasons, there is a need for a
mass spectrometry sample presentation apparatus which
provides a rapid, efficient method for analyzing
20   biomolecules using proven, advantageous MALDI matrices.

Summary of the Invention
        Accordingly, it is an object of the invention to
provide a sample presentation apparatus, mass
spectrometry method, and mass spectrometer that
25   overcome the disadvantages of the prior art.
        Another object of the invention is to provide an
improved sample presentation apparatus including a
complex capable of modifying biomolecules wherein it is
unnecessary to separate any reagent from the modified
30   biomolecule to prevent contaminating data obtained from
the modified biomolecule.
        Another object of the invention is to provide an
improved derivatized mass spectrometry sample
presentation apparatus which minimizes sample loss, and

thus works effectively with extremely small amounts of biomolecules.

Yet another object of the invention is to provide a mass spectrometry sample presentation apparatus which presents a complex capable of chemically modifying a biomolecule in a high effective concentration, and thus increases the rate of biomolecule modification reactions.

Another object of the invention is to provide a mass spectrometry sample presentation apparatus which digests and/or purifies biomolecules.

A further object of the invention is to provide a derivatized mass spectrometry sample presentation apparatus which can utilize existing optimized MALDI matrix systems.

Another object of the invention is to provide a mass spectrometry method wherein biomolecules are reacted with one reagent, and then a portion of the reacted biomolecules are reacted with a different reagent.

A further object of the invention is to provide a method for making a mass spectrometry sample presentation apparatus which overcomes the disadvantages of the prior art.

Yet another object of the invention is to provide a method of analyzing a biomolecule, wherein the biomolecule is exposed to the derivatized surface of the sample presentation apparatus and then incubated in a moist atmosphere. Subsequently, a MALDI matrix material is added, and finally the sample presentation apparatus is introduced into the mass spectrometer.

Another object of the invention is to provide a method of analyzing a polypeptide, wherein the molecular weight data is used to determine a limited amino acid sequence of the peptide. The limited amino acid sequence obtained by this method can then be used

to generate corr sponding nucleic acid sequences.
These nucleic acid sequences can then be used to search
a computer database containing known nucleic acid
sequences. A match may identify a known nucleic acid

5   sequence which codes for the polypeptide.
        A further object of the invention is to provide an
improved mass spectrometer which incorporates an
improved mass spectrometry sample presentation
apparatus.

10   These and other objects of the present invention
are obtained by providing a mass spectrometry sample
presentation apparatus comprising a mass spectrometry
sample presentation surface, wherein a complex is bound
to the surface which includes at least one molecule

15   which chemically modifies a biomolecule.
        A method of making a sample presentation apparatus
is further provided wherein a complex, which includes
at least one molecule which chemically modifies a
biomolecule, is bound to the sample presentation

20   apparatus surface.
        The objects of the present invention are further
accomplished through a method of analyzing a
biomolecule comprising the steps of:
        (a)   providing a mass spectrometry sample

25   presentation surface;
        (b)   binding at least one complex to the
                surface wherein the complex includes at
                least one molecule which chemically
                modifies a biomolecule;

30   (c)   contacting the biomolecule with the
                surface, thereby chemically modifying
                the biomolecule; and
        (d)   determining the molecular weight of the
                chemically modified biomolecule in a

35   mass spectrometer.

The objects of the present invention are further
obtained by a mass spectrometer. The mass spectrometer
has a sample presentation surface with a surface-bound
complex including at least one molecule which
5   chemically modifies a biomolecule. The mass
spectrometer also has a vacuum interlocking device for
introducing the surface into the machine, an apparatus
for volatilizing and ionizing the chemically modified
biomolecule, an electric field generator, a detector
10   and electronics for determining the molecular weight to
charge ratio of the modified biomolecule ions.

The sample presentation apparatus, method of
making a sample presentation apparatus, method of
analyzing biomolecules and mass spectrometer of the
15   present invention provide mass spectrometry information
for biomolecules which is both easier to obtain and of
higher quality, than was previously possible.

Brief Description of the Drawings

Fig. 1 is a MALDI mass spectrum of 10 picomoles of
20   chicken egg lysozyme digested for 10 minutes with
surface-bound trypsin.

Fig. 2 is a summary illustrating the correlation
between the calculated and observed masses for chicken
egg lysozyme fragments.

25   Fig. 3 is a MALDI mass spectrum of 1 picomole of
chicken egg lysozyme digested for 10 minutes with
surface-bound trypsin.

Fig. 4 is a MALDI mass spectrum of 1 picomole of
chicken egg lysozyme digested for 10 minutes with an
30   equimolar amount of unbound trypsin.

Fig. 5 is a MALDI mass spectrum of 2 μl of a
500 μM solution of chicken egg lysozyme digested with
an equal volume of slurried agarose immobilized
trypsin.

Fig. 6 is a summary of the residues of 1 picomole of chicken egg lysozyme obtained following a 10 minute digest with free trypsin and agarose-immobilized trypsin.

Fig. 7 is a schematic illustration of the amino acid sequence of α-cobratoxin.

Fig. 8 is a MALDI mass spectrum of undigested α-cobratoxin.

Fig. 9 is a MALDI mass spectrum of approximately 10 picomoles of α-cobratoxin digested for 30 minutes with surface-bound trypsin sample presentation apparatus.

Fig. 10 is a MALDI mass spectrum of approximately 10 picomoles of α-cobratoxin digested for 30 minutes with a surface-bound trypsin sample presentation apparatus in the presence of a reducing agent.

Fig. 11 is an exploded view of the MALDI mass spectrum of Fig. 10 in the m/z range of 1,000-5,000 Pa.

Fig. 12 is a positive ion MALDI mass spectrum of undigested human parathyroid hormone 1-34 (hPTH).

Fig. 13 is a MALDI mass spectrum of hPTH digested for 10 minutes on a surface-bound α-chymotrypsin mass sample presentation apparatus.

Fig. 14 is a MALDI mass spectrum of hPTH digested for 10 minutes on a surface-bound α-chymotrypsin sample presentation apparatus followed by pH adjustment to approximately 5 and addition of carboxypeptidase P (cpp). The inset illustrates the region of interest for final residue determination.

Fig. 15 illustrates the partial peptide sequence of hPTH generated by analyzing the mass spectrometry data from α-chymotrypsin and α-chymotrypsin/carboxypeptidase P (cpp) fragments.

Fig. 16 is a MALDI mass spectrum of hPTH digested for 5 minutes on a surface-bound trypsin sample presentation apparatus maintained at 45°C, pH 8.1.

Fig. 17 is a protein ladder sequence generated by
addition of cpp to the digested sample of Fig. 16.

Fig. 18 is a summary of the partial peptide
sequence generated by analyzing the mass spectrometry
5   data from trypsin and trypsin/cpp fragments.

Fig. 19 is an overlay of the mass spectra of
trypsin and trypsin/cpp fragments illustrating the use
of both the large-scale and ladder information to
extend the sequence determination.

10   Fig. 20 is a MALDI mass spectrum of hPTH digested
for 8 minutes on a surface-bound pepsin sample
presentation apparatus maintained at 45°C, pH 3.4.

Fig. 21 is a MALDI mass spectrum of hPTH digested
for 10 minutes on a surface-bound α-chymotrypsin mass
15   sample presentation apparatus maintained at 45°C, pH
3.4.

Fig. 22 is a MALDI mass spectrum of hPTH digested
for 10 minutes on a surface-bound α-chymotrypsin mass
sample presentation apparatus maintained at 45°C, pH
20   3.4, followed by a 10 minute digestion by surface-bound
cpp maintained at 35°C, pH 5.

Description of the Preferred Embodiments

The present invention provides a conventional mass
spectrometry sample presentation apparatus which has a
25   surface derivatized with complexes which chemically
modify biomolecules. This apparatus is typically
exposed to a biomolecule, and incubated to allow the
complex to modify the biomolecule. After incubation,
the modified biomolecule is preferably surrounded with
30   a MALDI matrix material and then introduced into a mass
spectrometer. The invention also include further
embodiments such as a mass spectrometer incorporating
such a sample presentation apparatus. Further
embodiments provide a method of performing mass

spectrometry, and of making a sample presentation apparatus.

As used herein, the term "derivatized" is meant to refer to a mass spectrometry sample presentation
5   apparatus having a bound complex. A derivatized sample presentation apparatus in accordance with the invention may be composed of any suitable material. The material can be a solid or liquid. Suitable solid materials include, but are not limited to insulators such as
10   quartz, semiconductors such as doped silicon and the like, and conductors including metals such as steel, gold and the like. Various insulating or conductive polymers may also be used. The surface of the sample presentation apparatus need not be made of the same
15   material as the rest of the apparatus. It is preferable for the surface to be clean so that a complex may adhere to the surface.

The complex attached to the presentation apparatus surface has two distinct functionalities. As used
20   herein, the term "complex" refers to a chemical which includes at least one molecule which chemically modifies a biomolecule. The complex has a "tethering" function, wherein it is capable of attaching itself at at least one end to the surface. Moreover, it also has
25   a "reactive" function wherein it is capable of chemically modifying the analyte. As used herein, the term "chemically modify" is meant to refer to purification or digestion of an analyte, but not binding an analyte. The complex may further comprise a
30   tethering molecule which binds to the surface, and a reactive molecule which binds to the tethering molecule and modifies a biomolecule.

Typically, the "tethering" function is associated with one group of atoms (_i.e._, the tethering molecule)
35   while the "reactive" function is associated with a different group of atoms (_i.e._, the reactive molecule).

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The complex may comprise both a tethering molecule
which binds to the sample presentation surface and a
reactive molecule which binds to the tethering molecule
and modifies a biomolecule. However this separation of
function is not necessary. The tethering molecule is
preferably selected from the class of molecules which
has proven to be effective as an immobilized ligand.
Many specific examples of such molecules are listed on
pages T-156 to T-200 of the 1995 Pierce Corporation
Catalog (Pierce Corp., P.O. Box 117, Rockford Il.
61105). Suitable tethering molecules include:
dithiothreitol, dimethyladipimidate-2*HCL,
dimethylpimelimidate*HCL, dimethylsuberimidate*2HCL,
dimethyl 3,3'-dithiobispropionimidate*2HCL,
disuccinimidyl glutarate, disuccinimidyl suberate, bis
(sulfosuccinimidyl) suberate,
dithiobis(succinimidylpropionate),
dithiobis(sulfosuccinimidylpropionate), ethylene
glycobis (succinimidylsuccinate), ethylene
glycobis(sulfosuccinimidylsuccinate), disuccinimidyl
tartarate, disulfosuccinimidyl tartarate, bis[2-
(succinimidyloxycarbonyloxy)ethyl]sulfone, bis[2-
(sulfosuccinimidooxy-carbonyloxy)ethyl]sulfone,
succinimidyl 4-(N-maleimido-matyl) cyclohexane-1-
carboxylate, sulfo-succinimidyl 4-(N-maleimidomethyl)
cyclohexane-1-carboxylare, m-Maleimidobenzoyl-N-
hydroxysuccinimide ester, m-maleimidobenzoyl-N-
hydroxysulfosuccinimide ester, succinimidyl 4-p-
maleimido-phenyl)-butyrate, sulfosuccinimidyl 4-(p-
maleimidophenyl)-butyrate, bismaleimidohexane, N-(λ-
maleimidobutyryloxy)succinimide ester, N-(λ-
maleimidobutyryloxy)sulfosuccinimide ester, n-
succinimidyl(4-iodoacetyl)aminobenzoate,
sulfosuccinimidyl(4-iodoacetyl)-aminobenzoate, 1,4-di-
[3'-2'-pyridyldithio(propionamido)butane], 4-
succinimidyl-oxycarbonyl-α(s-pyridyldithio)toluene,

sulfosuccinimidyl-6-[α-methyl-α-(2-pyridyldithio)-
toluamido]hexanoate, n-succinimidyl-3(2-pyridyldithio)-
propionate, succinimidyl 6-[3-(2-pyridyldithio)-
propionamido]hexanoate, sulfosuccinimidyl-6-[-3-(2-
5   pyridyldithio)-propionamido]hexanoate,
sulfosuccinimidyl-6-[3-(2-pyridyldithio)-
propionamido]hexanoate, 3-(2-pyridyldithio)-propionyl
hydrazine, 1-ethyl-3-(3-dimethylaminopropyl)-
carbodiimide hydrochloride, n,n'-
10   dicyclohexylcarbodiimide, 4-(p-azidosalicylamido)-
butylamine, azidobenzoyl hydrazine, N-5-azido-2-
nitrobenzoyloxysuccinimide, n-5-azido-2-
nitrobenzoyloxysuccinimide, n-[4-(p-
azidosalicylamido)butyl]-3'(2'-
15   pyridyldithio)propionamide, p-azidophenyl glyoxal
monohydrate, 4-(p-azidosalicyl-amido)butylamine, 1-(p-
azidosalicylamido)-4-(iodoacetamido)butane, bis-[β-4-
azidosalicylamido)ethyl]disulfide, n-
hydroxysuccinimidyl-4-azidobenzoate, n-hydroxysulfo-
20   succinimidyl 4-azidobenzoate, n-hydroxysuccinimidyl-4-
azidosalicylic acid, n-hydroxysulfosuccinimidyl-4-
azidosalicylic acid, sulfosuccinimidyl-(4-
azidosalicylamido)-hexanoate, p-nitrophenyl-2-diazo-
3,3,3-trifluoropropionate, 2-diazo-3,3,3,-trifluoro-
25   propionate, n-succinimidyl-(4-azidophenyl)1,3/-
dithiopropionate, sulfosuccinimidyl-(4-
azidophenyldithio)propionate, sulfosuccinimidyl-2-(7-
azido-4-methylcoumarin-3-acetoamide)ethyl-1,3'-
dithiopropionate, sulfosuccinimidyl 7-azido-4-
30   metylcoumarin-3-acetate, sulfosuccinimidyl 2-(m-azido-
o-nitrobenzamido)-ethyl-1,3'-dithiopropionate, n-
succeinimidyl-6-(4'-azido-2'-nitrophenyl-
amino)hexanoate, sulfosuccinimidyl 6-(4'-azido-2'-
nitrophenylamino)hexanoate, sulfosuccinimidyl 2-(p-
35   azidosalicylamido)ethyl-1,3'-dithiopropionate,
sulfosuccinimidyl 4-(p-azidophenyl)-butyrate. Various

protocols for using such ligands are explained in
"Immobilized Affinity Ligand Techniques" by Greg T.
Hemanson et al. (1992), which is incorporated herein by
reference.

5   The complex may chemically modify a biomolecule
analyte in several different ways. As used herein, the
term "biomolecule" is meant to refer to DNA, RNA,
polypeptides, carbohydrates and combinations thereof.
For example, the complex may digest, or purify the

10   biomolecule. As used herein, the term "digest" is
meant to refer to chemical or enzymatic cleavage of a
biomolecule into fragments and the term "purify" means
to remove undesirable materials from a solution
containing the biomolecule. As used herein, the term

15   "fragment" is meant to refer to a part of a biomolecule
which has been cleaved from the rest of the biomolecule
using a chemical or enzymatic reagent. Several
different reactive molecules are available for
digesting biomolecules. If the biomolecule is a

20   polypeptide, the complex will preferably comprise a
chemical cleavage agent or an enzymatic protease.
          Enzymatic proteases are specific polypeptides
which cleave polypeptides. Proteases may cleave
themselves by a process known as autolysis. Several

25   enzymatic proteases cleave polypeptides between
specific amino acid residues. Examples of proteases
which cleave nonspecifically include subtilisin, papain
and thermolysin. Examples of proteases which cleave at
least somewhat specifically include: aminopeptidase-M,

30   carboxypeptidase-A, carboxypeptidase-P,
carboxypeptidase-B, carboxypeptidase-Y, chymotrypsin,
clostripain, trypsin, elastase, endoproteinase Arg-C,
endoproteinase Glu-C, endoproteinase Lys-C, factor Xa,
ficin, pepsin, plasmin, staphylococcus aureus V8

35   protease, proteinkinase K and thrombin.

Chemical cleavage agents which cleave polypeptides
between specific amino acid residues include cyanogen
bromide, O-iodosobenzoate or O-iodosobenzoic acid,
dilute hydrochloric acid, N-bromosuccinimide, sodium

5   hydrazine, lithium aluminum hydride, hydroxylamine and
2-nitro-5-thiocyanobenzoate. Suitable chemical
cleavage agents also include Sanger's Reagent (2,4-
dinitrofluorobenzene), Edmund's Reagent
(Phenylisothiocyanate), and immobilized derivatives of

10   these Reagents. Moreover, coordinated transition metal
complexes such as the tetradentate Co (III) complex,
β-[Co(triethylenetetramine)-OH(H_2O)], may also be used
as effective cleavage agents. See Buckingham et al. 89
J.A.C.S. 1082 (1967).

15   When the biomolecule to be analyzed is a
polypeptide, it may be advantageous to add a reducing
agent to sever any disulfide bonds between the amino
acids. Severing these bonds will result in further
fragmentation of the peptide, thus yielding more

20   information about peptide structure. Disulfide
reduction agents may be bound onto the sample
presentation surface, or used in solution. Suitable
disulfide reducing agents include β-mercaptoethanol,
cysteine, and dithiothreitol (DTT). Carboxypeptidase-

25   P, dithioerythritol (DTE) lipomide and N-
acetylhomocysteine can be used as surface-bound
disulfide reduction agents.

For biomolecules such as DNA or RNA, it may be
advantageous to add a restriction endonuclease or

30   exonuclease to the sample presentation apparatus
surface. These enzymes can cleave DNA and RNA between
specific nucleases, creating fragments of DNA or RNA.
Specific restriction endonucleases and exonucleases are
well known to those skilled in the art. Examples of

35   enzymes which cleave both DNA and RNA include, but are
not limited, to snake-venom phosphodiesterase and

spleen phosphodiesterase. Enzymes which cleave only
DNA include, but are not limited to, deoxy-ribonuclease
I and II. Ribonuclease I (pancreas) and T. (mold)
cleave RNA.

5   When analyzing a biomolecule containing
carbohydrate, it may be advantageous to add fucase, O-
or N-glycanase, mannase, neuraminidase, galactosidase
or glucosidase to the surface. These enzymes will
generate carbohydrate fragments of specific molecular
10   weight which can be determined in the mass
spectrometer.

       The complex attached to the sample presentation
surface may also purify the biomolecule. For example,
the complex may comprise a reactive molecule which
15   invisibly binds common impurities such as detergents,
lipids, endotoxins or proteases.

       The present invention also provides a method for
making the above-described derivatized mass
spectrometry sample presentation apparatus. In this
20   method it is essential that a complex which modifies
biomolecules be bound to the surface of the sample
presentation apparatus. In one embodiment, the entire
complex is first synthesized or purified, and then
reacted with the surface. In an alternate preferred
25   embodiment, the complex is formed by first reacting the
sample presentation surface with a tethering molecule,
and then later reacting the tethered molecule with a
reactive molecule.

       Another embodiment of the present invention
30   provides a method for analyzing a biomolecule.
Specifically, the method involves: (1) providing a mass
spectrometry sample presentation surface, (2) binding
at least one complex on the surface wherein the complex
includes at least one molecule which chemically
35   modifies a biomolecule, (3) contacting the biomolecule
with the surface, thereby chemically modifying the

biomolecule (4) applying a MALDI matrix to the surface
and (5) determining the molecular weight of the
biomolecule in a mass spectrometer. The biomolecule
may be contacted with the surface while in solution or
5   in the vapor phase.

    The contacting step is preferably performed above
room temperature, most preferably up to 55°C, to speed
the reaction rate. However, it should not be performed
at a temperature high enough to cause degradation of
10 the analyte or the complex. For certain complexes, it
may be advantageous to perform the contacting step
below room temperature, most preferably above -10°C.
This contacting step is allowed to proceed for a
sufficient time, typically 5-30 minutes. The actual
15 reaction time will depend on the particular complex and
biomolecule. When proteases are used to digest
polypeptide biomolecule, the contacting step is
preferably performed in a moist atmosphere. Such an
atmosphere may be created for example, by providing a
20 water reservoir and enclosing it along with the sample
presentation surface and biomolecule in a closed
system.

    Next, a MALDI matrix is added to the reacted
biomolecule. The MALDI matrix may be any material
25 which solubilizes biomolecules, absorbs light energy at
a frequency easily accessible by a laser, and is
unreactive with respect to biomolecules. Suitable
matrices include nicotinic acid, pyrozinoic acid,
vanillic acid, succinic acid, caffeic acid, glycerol,
30 urea or tris buffer (pH 7.3). Preferable matrices
include α-cyano-4-hydroxycinnamic acid, ferulic acid,
2,5-dihydroxybenzoic acid, sinapic (or sinapinic) acid,
3,5-dimethoxy, 4-hydroxy-trans-cinnamic acid, other
cinnamic acid derivatives, gentisic acid and
35 combinations thereof.

A further embodiment of the present invention
provides a mass spectrometer which incorporates the
above-described sample presentation apparatus. The
mass spectrometer according to the present invention
5   further comprises a vacuum interlocking device for
introducing the sample presentation apparatus into a
volatilization chamber, an apparatus for volatilizing
and ionizing the analyte, an optional electric field
generator which is oriented to cause biomolecule ions
10   to travel toward a detector, and electronics for
determining the molecular weight to charge ratio for a
particular ion based on its trajectory.

     This mass spectrometer is compatible with all of
the techniques normally used for volatilizing and
15   ionizing analytes. Such techniques include, but are
not limited to, electrospray ionization, Californium^{252}
plasma desorption, field desorption, fast atom
bombardment, liquid secondary ion MS, laser desorption,
and thermospray ionization. Preferably, the MALDI
20   technique is used for volatilizing and analyzing
biomolecules. The mechanical structures or equipment
required in these techniques are well known to those
skilled in the art.

     The spectrometer usually contains an electric
25   field generator which has electrically charged metal
structures between the area in the spectrometer where
the ions are generated and the detector. These
structures must be designed such that they attract
ions, but the ions can travel past them to reach the
30   detector. Preferably, the structures are charged metal
grids with apertures that allow some of the ions to
pass through and drift toward the detector. The
structures must be located between the ion generation
area and the detector. They must also have a potential
35   difference which acts to accelerate ions toward the
detector.

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Some mass spectrometers also use magnetic fields
to deflect ions as they travel towards the detector.
Such instruments determine the mass of an ion based on
its deflection in the magnetic field. It is, however,
5   preferable to use a time-of-fight mass spectrometer to
determine the mass of biomolecules.

The electronics for determining the molecular
weight to charge ratio of an ion based on its
trajectory typically includes an ion impact detector,
10   data-acquisition electronics, and data analysis
electronics, such as a computer.

Many types of detectors are compatible with the
present invention. Some specific examples of detectors
include the Faraday cup, electron multipliers, electro-
15   optical ion detectors and photographic emulsions.

Specific embodiments in accordance with the
present invention will now be described in detail.
These examples are intended to be illustrative, and the
invention is not limited to the materials, methods or
20   apparatus set forth in these embodiments.

Example 1

Preparation of Sample Presentation Apparatus

Circles of gold (Au) foil (99.9%), available from
Johnson Matthey, 2.5 mm diameter and 0.01 mm thick were
25   punched out and placed in a polyethylene
microcentrifuge tube. Care was taken to avoid any
contamination of the gold foil circles. The gold foil
was activated according to the method of Katz. See,
Katz, E.Y., J. Electroanal. Chem., Vol. 291, p. 257
30   (1990). The circles were treated with a crosslinking,
or tethering molecule, solution of Dithiobis
[succinimidylpropionate] (DSP) available from Pierce
dissolved in isopropanol to approximately 0.1M. The
DSP/isopropanol solution was essentially saturat d at
35   this concentration. The circles were treated with the

DSP/isopropanol solution for fifteen minutes, with
occasional mixing using a vortex mixer. This tethering
molecule solution was poured off, and the gold circles
were rinsed repetitively with pure isopropanol,

5   followed by three rinses with pure ethanol. The gold
circles were vacuum dried and transferred to new
microcentrifuge tubes prior to treatment with a
reactant molecule or biomolecule.

                          Example 2
10   Preparation of Sample Presentation
             Apparatus with Surface-Bound Trypsin
        A mass spectrometry sample presentation apparatus
was prepared by binding trypsin to the surface of the
apparatus as follows. Trypsin type XIII: tosyl-L-

15   phenylalanine chloromethyl ketone (TPCK) treated, from
bovine pancreas available from Sigma, was dissolved in
20 mM phosphate buffer, pH 7.8 to a concentration of 1
mg/mL for use in preparation of the derivatized sample
presentation apparatus. To attach the enzyme to the

20   crosslinker or tethering molecule on the gold foil
circles prepared in Example 1, the circles were
incubated with a 1 mg/mL solution of enzyme in 20 mM
phosphate buffer, pH 7.8 overnight in a refrigerator
with occasional agitation. Following incubation, the

25   gold foil circles were rinsed vigorously with the
phosphate buffer, and subsequently with a 0.1% aqueous
solution of Triton-X100, using a vortex mixer for both
rinses. The gold foil circles were then vacuum dried
and stored in polyethylene microcentrifuge tubes.

30   Physical attachment of the gold foil circle surfaces to
the stainless steel sample presentation apparatuses
conventionally used for routine MALDI analysis was
accomplished by application of a small amount of spray
adhesive to the conventional sample presentation

35   surface or probe tip, which was then pressed onto one

of the gold foil circl s. Prior to attachment, the
conventional stainless steel (304) mass spectrometer
sample presentation apparatuses were electropolished
and thoroughly rinsed in deionized water followed by
5   ultrasonic cleaning in methanol. Even pressure was
maintained on the probe tip and gold foil while the
adhesive was allowed to bond. The sample presentation
apparatus with bound trypsin was then used for protein
digestion as described hereinbelow. In practice,
10   multiple tips were made and stored in polyethylene
tubes at room temperature until needed.

                          Example 3
                 Au/trypsin Sample Presentation
                 Surface Contacted with Lysozyme
15   Digestions were performed with the Au/trypsin
active surfaces prepared in Example 2 by the
application of aliquots (either 4μL or 400 nL) of 250
nM lysozyme hen egg solution (dissolved in 20 mM
phosphate; 10 mM dithiothreitol (DTT); pH 8.1 buffer)
20   directly to the sample presentation apparatus surfaces.
The surfaces were allowed to stand in a humid enclosure
maintained at 40°C. The solution volume on the surface
was monitored, and additional aliquots of 1-2 μL of
phosphate buffer were added if the volume of liquid
25   appeared to be less than 2 μL. After 10 minutes the
apparatus was removed from the humid environment, 2 μL
of the MALDI matrix, α-cyano-4-hydroxycinnamic acid
(prepared as a saturated solution in a solvent mixture
of 2:1, 1.3% aqueous trifluoroacetic acid:acetonitrile
30   (ACCA)), was applied and the surface was allowed to air
dry.

Comparative Example 4
Protein Digestion/Sample
Preparation with Free Trypsin

A 1:1 mole ratio of trypsin to lysozyme was
prepared by addition of 2 μL of 500 nM lysozyme with
2μL of 500 nM trypsin (both prepared in a 20 mM
phosphate; 10 mM DTT; pH 8.1 buffer). The combined
volume was placed in a 600 μL microcentrifuge tube and
incubated at 40°C for 10 minutes. The reaction was
halted by the addition of 2 μL of the MALDI matrix ACCA
and the total volume of the digest/matrix mixture was
placed on an inert mass spectrometer sample
presentation apparatus and allowed to air dry.

Comparative Example 5
Agarose Immobilized Trypsin

A 1:1 volume ratio of lysozyme to agarose/trypsin
was prepared by addition of 2 μL of 500 nM lysozyme
with 2μL of slurried agarose/trypsin (both prepared in
a 20 mM phosphate; 10 mM DTT; pH 8.1 buffer). The
slurried agarose contained approximately 1 μL of beaded
reagent. The combined volume was placed in a 600 μL
microcentrifuge tube and incubated at 40°C for 10
minutes. The reaction was halted by the addition of 2
μL of ACCA matrix and the total volume (with beaded
reagent) of the digest/matrix mixture was placed on an
inert mass spectrometer conventional sample
presentation apparatus or probe tip and allowed to air
dry.

Example 6
Time-of-Flight Mass Spectrometry

Mass spectrometry was performed using the sample
presentation apparatus prepared in Example 3,
Comparative Example 4, and Comparative Example 5 using
a linear time-of-flight mass spectrometer. Desorption

and ionization were accomplished using the third
harmonic (355 nm) output of the Nd:YAG laser (Continuum
Surelite I). The optical path of the laser contained a
variable attenuator (Newport 935), a right-angle beam
5   steering prism and a 250 mm focal length quartz lens
placed approximately 300 mm from the sample target.
Ions generated from the matrix/analyte sample were
accelerated to a potential of 30 kV using a two-stage
acceleration source (total distance = 1.5 cm). An
10   electrostatic particle guide was used to assist in ion
transmission over the flight path. Detection was
accomplished using a hybrid, microchannel-
plate/discrete dynode, electron multiplier. Thresholds
of ionization were determined empirically by increasing
15   the intensity of the laser light at the sample surface
while monitoring the ion signal real-time with a 50 Mhz
oscilloscope (Tektronix TDS 310). Upon observation of
stable ion signal, 50 laser shots were signal averaged
using a 500 MS/s digital storage oscilloscope
20   (Tektronix TDS 520A) and transferred to an IBM-
compatible 486 computer. The data were analyzed using
the PC compatible software LabCalc (Galactic
Industries). All mass spectra were obtained in the
positive-ion mode. External mass calibration was
25   accomplished with the single and double-charged peaks
of bovine insulin (MW = 5,733.5 Da). Masses of digest
fragments were calculated using the known hen egg
lysozyme amino acid sequence and the peptide data
manipulation routine PROCOMP. This program is publicly
30   available from Dr. Philip C. Andrews, University of
Michigan Medical Center, Ann Arbor, Michigan 48104.

Prior to enzymatic cleavage, the integrity of the
lysozyme samples was verified through MALDI analyses
using sample presentation apparatus without any bound
35   complex. No signals other than those due to the
lysozyme ions, singly- and multiply-charged signals,

were observed during these analyses. Fig. 1 shows the
results of a 10 minute digestion (40°C) of 10 picomoles
of lysozyme using the sample presentation apparatus
with trypsin bound to the gold surface as described in
5   Example 3. No signals were observed over m/z = 6,000,
which indicates high trypsin activity. Ion signals are
observed for 33 out of 67 possible digest products (in
the mass range up to 6,000 Da). Signals below m/z =
1,000 Da were generally not considered because of
10   possible interferences from the matrix. Signal
intensity and quality was such to allow laser
desorption conditions capable of resolving tryptic
fragments at m/z = 1,424.8, 1,429.6 and 1,435.5 Da
(inset). Fig. 2 shows the correlation between the
15   calculated masses of the enzymatically cleaved
fragments and those observed in the mass spectrum.

Fig. 3 shows the mass spectrum obtained from using
the same procedure performed with only 1 pmol of
lysozyme applied to the surface. Virtually the same
20   fragmentation pattern is observed, with an expected
sacrifice in the signal-to-noise ratio of the data.

For comparison, 1 pmol of lysozyme was digested
using either an equal mole ratio of free trypsin or an
equal volume of agarose immobilized trypsin as
25   described in Comparative Examples 4 and 5. Results of
the digestion using free trypsin are shown in Fig. 4.
Many of the low mass signals compliant with trypsin-
generated fragments are observed which are shown in
Fig. 6. Strong signals at m/z 1177, 1459, 1498, 2166,
30   2191, and 2275, however, are attributed to autolysis
products of the trypsin since these fragments are also
observed during the MALDI mass analysis of trypsin as
shown in Fig. 6.

Additionally, trypsin whole molecule signals
35   (singly- and multiply- charged signals) also appear in
the spectrum. The autolysis and parent signals

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contaminate the data because these fragments are
incorporated in the matrix. The trypsin used in
digestion is not bound to the surface, and therefore,
there is nothing to prevent the trypsin molecular ion

5   and autolysis products from incorporating into the
matrix crystals. In addition, a decrease in the
signal-to-noise ratio is also observed, relative to
Fig. 3, and is attributed to loss of sample in transfer
and handling.

10   Agarose-immobilized trypsin was used in order to
attempt to reduce the background contribution due to
the trypsin autolysis products. The results obtained
from the 10 minute, 40°C digestion of 1 pmole of
lysozyme using approximately 1 µL of beaded agarose as

15   described in Comparative Example 5 are shown in Fig. 5.
Signals are observed for nine lysozyme digest
fragments, and only two autolysis product peaks at m/z
2164 and 3371 are found. In contrast to both the
derivatized sample presentation apparatus and the free

20   trypsin digestions, few peaks corresponding to digest
fragments are observed. However, a marked decrease in
the overall signal intensity is observed. Since the
agarose is a porous medium, it is believed that digest
fragments are lost through a combination of

25   incorporation into the media, slow digestion due to
poor localization of analyte around the immobilized
enzyme, and inefficient elution using a small volume of
matrix.

Comparing the spectrum obtained using the

30   Au/trypsin derivatized sample presentation apparatus
with those of the immobilized and free trypsin digests,
it is clear that digests performed using the
derivatized sample presentation apparatuses are
noticeably free of background signals due to enzyme

35   autolysis products and maintain a high level of
activity. It is estimated from the digest pattern

which indicates the degree of total digestion that the
activity of the derivatized surfaces in accordance with
the invention falls somewhere between that of the
agarose-immobilized and free trypsin (approximately

5   0.05 and 0.15 TAME units, respectively). This level of
derivatized surface activity has been found to remain
reasonably consistent from batch to batch if similar
preparation conditions, e.g., incubation time,
temperature, and buffer, are used. However, activity

10   has been found to be seriously impaired if the surfaces
are inadvertently touched resulting in deposition of
oils, or contaminated with adhesive during the fixing
of the Au/trypsin foil to the conventional sample
presentation apparatus. Care must be taken in the

15   final steps of surface preparation. Alternatively, the
risk of contamination has been completely eliminated by
sputter-coating the surfaces with gold and coupling
trypsin directly to this gold coating. Trypsin
activity using this method was found to be

20   approximately equal to that of the gold foil method.

                           Example 7

                 Au/trypsin Sample Presentation
                Surface Contacted with α-cobratoxin
          α-cobratoxin was analyzed using an immobilized

25   trypsin sample presentation apparatus as described in
Example 2.
          First, the unfragmented α-cobratoxin was analyzed
using the MALDI method. Signals were obtained which
correspond the singly and doubly charged ions of the

30   intact peptide. As can be seen from Fig. 7 and Fig. 8,
singly and doubly charged ions corresponding to residue
1-71 are observed at 7,823 and 3,912 Da, respectfully.
Signals less than m/z approximately 1,000 Da are due
to the ACCA matrix.

Next 10 picomoles of α-cobratoxin were digested
for 30 minutes with a trypsin surface prepared
according to Example 2. The α-cobratoxin was applied
to the surface in a 3 μL volume of 10 mM phosphate
5   buffer (pH 7.8). The reaction was allowed to proceed
in a highly humid environment, at room temperature, for
30 minutes before being stopped by the addition of an
equal volume of ACCA matrix. The mixture was allowed
to dry before being inserted into the mass
10   spectrometer. The resulting mass spectrum, shown in
Fig. 9, shows singly and doubly charged ion signals for
peptides with molecular weights of 7,445, 7,575 and
7,823 Da. These weights correspond to peptide
sequences 1-68, 1-69 and 1-71, respectively, as shown
15   in Fig. 7. These are the primary fragments expected
from the tryptic treatment of the non-reduced peptide.
Five other cleavage sites are possible, each producing
different molecular weight fragments. However, these
sites are stearically hindered and not available to
20   trypsin. Therefore, these five other possible peaks
were not observed.

α-cobratoxin was further analyzed by repeating the
above-described 10 picomole α-cobratoxin digestion by
surface-bound trypsin in the presence of a reducing
25   agent (10 pmol α-cobratoxin in 3 μL - 10 mM phosphate;
10 mM DTT). As can be seen from Fig. 7, the reducing
agent broke the disulfide bonds, thus enabling the
generation of more fragments. The resulting spectrum
shown in Figs. 10 and 11 shows a set of peptide
30   fragments indicative of reduced cobratoxin.

Example 8

Preparation of Sample Presentation Apparatus with Surface-Bound α-Chymotrypsin

A mass spectrometry sample presentation apparatus
35   was prepared by binding α-chymotrypsin to the surface

of the apparatus as follows. α-chymotrypsin or TPCK-
treated α-chymotrypsin was dissolved in 20 mM phosphate
buffer, pH 7.8 to a concentration of 1 mg/mL for use in
preparation of a derivatized sample presentation
5   apparatus as described in Example 2.

Example 9
Sequence Determination For Design of
Nucleic Acid Probes And Gene Identification
The combination of surface-immobilized and free-
10   peptidases were used to generate heterogeneous fragment
mixtures from an original peptide as follows.
Enzymatically active trypsin and α-chymotrypsin
mass spectrometry sample presentation surfaces were
prepared as described in Examples 2 and 8.
15   The synthetic peptide human parathyroid hormone
1-34 (hPTH) was chosen as a test peptide for analyses.
The sequence of the peptide is:

          10   20   30
     SVSEIQLMHN LGKHLNSMER VEWLRKKLQD VHNF

20   Digestions were performed in the following manner.
Three microliter aliquots of a 0.01 mg/mL solution of
hPTH (in a buffer of 20 mM NaHPO$_4$, pH 8.1) were applied
to derivatized mass spectrometer sample presentation
apparatus prepared in accordance with Example 2 or
25   Example 8. The digestion was carried out in a high
humidity environment at a temperature of between 40 and
50°C. The digestions were allowed to run for 5 to 10
minutes before 0.5 -1.0 μL aliquots of the reaction
mixtures were removed from the surfaces. These
30   aliquots were MALDI analyzed for endopeptidase mapping.
The pH was reduced to approximately 5 with the addition
of an aliquot of 20 mM NH$_4$COOCH$_3$, the volume of which

was equal to that of the remaining reaction mixture.
500 nL aliquots of 0.001 mg/mL carboxypeptidase P
(CPP), in the acetate buffer, were then added to the
reaction mixtures. Protein ladder digestions were
5   allowed to run for between 1 to 3 minutes before the
surfaces were removed from the heated high humidity
environment and the reactions halted through the
addition of an equal volume of MALDI matrix. The
matrix/reaction mixture was allowed to air-dry and the
10   enzymatically-active surfaces inserted directly into
the mass spectrometer.

Fig. 12 is an ACCA-MALDI mass spectrum of 2.5
picomoles of the hPTH, the starting point of reference
for the following enzymatic analyses. A strong singly-
15   protonated signal is observed at a mass-to-charge ratio
(m/z) of 4,119.0 Da, a doubly- charged at
m/z = 2,019.7 Da, and little else other than matrix ion
signals at m/z < 500 Da.

The hPTH was further analyzed after a 10 minute
20   digestion on an Au/α-chymotrypsin surface maintained at
40°C, pH 8.1. The results of this digestion are shown
in Fig. 13. Four major signals at 1,398.5, 1,706.2,
1850.0 and 2,738.7 Da are observed as shown in Fig. 13.

Before adding the MALDI matrix to obtain the mass
25   spectrum shown in Fig. 13, a portion of the digested
peptide was transferred to a second conventional sample
presentation apparatus surface. The portion of the
digested peptide on the second conventional sample
presentation surface was adjusted to a pH of
30   approximately 5 and further exposed to cpp. Fig. 14
shows the MALDI mass spectrum obtained from this second
sample. A series of ion signals due to cpp digestion
is observed in the m/z range of 1,800 - 2,800 Da,
presumably stemming from the Au/α-chymotrypsin digest
35   signal at m/z = 2,738.7. The first residue is a
preferred cleavage site for α-chymotrypsin. Fig. 15

summarizes the masses of the signals observed in Fig.
14, the differences in mass between adjacent signals
and the residues determined from the differences. The
partial sequence (read as shown in Fig. 15 from N to C-
5   terminal is determined by sequential mass difference to
give (pep) - MERVEW - COOH.

A second similar analysis of the hPTH was
performed using an Au/trypsin sample presentation
surface followed by cpp digestion. Fig. 16 shows the
10   results of the Au/trypsin digestion after 5 minutes at
a temperature of 45°C (pH 8.1). Fig. 18 summarizes the
masses observed for the digestion. The trypsin
digestion shown in Fig. 16 resulted in the production
of several starting fragments, and indicated the
15   presence of the short sequence (Arg/Lys) - Lys - Lys.

Before adding the MALDI matrix to obtain the mass
spectrum shown in Fig. 16, a portion of the digested
peptide was transferred to a second, conventional
sample presentation apparatus surface. The cpp was
20   then added to this second portion. After pH adjustment
to approximately 5, the ladder digestion was allowed to
proceed for 3 minutes before being terminated by the
addition of ACCA to the sample presentation surface.
Fig. 17 shows the resulting sequence ladder. From the
25   observed mass differences summarized in Fig. 18 the
sequence was determined to be (pep) - NSMERVEW(L/I)RK -
COOH. The first residue cleaved by cpp is that of Arg.
Arg is a preferred cleavage site for trypsin. This is
obviously an extension of the partial sequence
30   determined using Au/α-chymotrypsin/cpp.

The large scale sequence, (Arg/Lys) - Lys -Lys,
overlaps with the ladder sequence at correct mass.
This allows both spectra to be combined for sequence
extension by an additional Lys. The first residue
35   observed in the Au/α-chymotrypsin/cpp series, from the
C-terminal end, was determined to be tryptophan. This

is expected because tryptophan is one of the preferred
sites of α-chymotrypsin cleavage.

There are two ion signals to chose from in the
region of the last cleavage of the Au/α-

5   chymotrypsin/cpp digestion as can be seen from Fig. 15,
inset, leading to the possibility of an ambiguity. The
two mass differences represent the possibility of
either a serine or a proline as the final residue. On
the basis of the mass assignment error observed in the

10   entire series (average approximately 0.15 Da), the
final determination of serine, with -0.1 Da error was
made over that of proline (with -2.2 Da error). The
resulting sequence was determined to be (pep) - SMERVEW
- COOH.

15   A nominal mass difference of 128 Da was observed
at several points in the spectrum resulting from the
initial trypsin cleavage in the Au/trypsin/cpp
analysis. Considering that trypsin will cleave only at
lysine or arginine, the 128 Da mass difference is

20   highly likely to result from a lysine C-terminal to
either another lysine or an arginine. Likewise, two
adjacent mass differences of 128 Da, as observed in the
3,000 to 3,300 Da region, indicates the presence of
Lys-Lys in the sequence, with the third residue being

25   either another lysine or an arginine. This
information, in combination with recognizing overlaps
in the endo- and exopeptidase spectra, allows the two
spectra to be used in combination to extend the
sequence determination. Fig. 19 illustrates a ladder

30   sequence extended by an additional lysine determined
from the (Arg/Lys) - Lys-Lys observed in the trypsin
spectrum overlapping with the Arg-Lys of the ladder
sequence. The 128 Da mass differences observed in this
manner can be unambiguously assigned to Lys as opposed

35   to Gln which has the same molecular weight. The result

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is that 12 residues of the sequence were determined to
be (pep) -NSMERVEW(L/I)RKK - COOH.

In gathering this data, four 50 to 100 laser-shot
mass spectra, with quality and signal-to-noise great
5   enough to determine relative molecular masses within
approximately 0.2 Da, were generated using a total of
approximately 15 picomoles of sample. Typical of the
MALDI process, the sample preparations were capable of
thousands of single laser-shot mass spectra of equal
10   quality. As no special efforts were taken to minimize
the amount of sample applied to the sample presentation
surface apparatus, and the surface area of the
apparatus was quite large, it may be possible to reduce
the analyte sample amount, without sacrificing quality,
15   by simply downscaling the whole process by reduction of
surface area and application of less analyte sample.

It was important to maintain the derivatized
sample presentation surfaces at elevated temperatures
in order to perform these digestions rapidly. The
20   temperatures used for both the endo- and exopeptidase
digestions, performed at elevated temperatures of up to
approximately 55°C, are somewhat above that typically
used in proteolytic digestions, namely, 37°C. This,
combined with the high relative activity of the
25   enzymatically-active sample presentation surfaces, is
believed to be responsible for the rapid analysis
times. Although not guaranteed in every case, the 15
minute analysis time demonstrated in hPTH example
represents a rate of analysis of just under one residue
30   per minute.

After a peptide sequence is determined by the
above-described process, the rules of degeneracy can be
used to identify many nucleic acid sequences which code
for the peptide sequence. See, e.g., Biochemistry,
35   Stryer, pp. 91-115 (1988). Since many different
nucleic acid sequences code for a single peptide,

several nucleic acid sequences can be generated from
the peptide sequences. The nucleic acid sequences can
be used to search a database of nucleic acid sequences,
e.g., Genbank, to identify genes which code for a
5   particular polypeptide.

Example 10
Preparation of Sample Presentation
Apparatus with Surface-Bound Pepsin
     A mass spectrometry sample presentation apparatus
10   was prepared by binding pepsin to the surface of the
apparatus as follows. Gold foil was first activated
with DSP according to the method given in Example 1.
The DSP activated gold was then reacted with ethylene
diamine (20% v/v in isopropanol) at room temperature
15   for 30 minutes. After incubation, the gold foil was
rinsed thoroughly with isopropanol and then reacted
with 0.1 M[1-ethyl-3-(3-
dimethylaminopropyl)carbodiimide] in 20 mM phosphate
buffer at pH 4.5, for 30 minutes at room temperature.
20   The foil was then rinsed thoroughly with the phosphate
buffer. Next, a solution of 10 mg/ml pepsin in
phosphate buffer was added to the surface and allowed
to incubate for 4 hours at 4°C. After incubation the
foil was rinsed repeatedly with phosphate buffer and
25   finally vacuum dried.

Example 11
Au/pepsin Sample Presentation
Surface Contacted with Pepsin
     A digestion was performed with the Au/Pepsin
30   active surfaces prepared in Example 10 by the
application of approximately 5 pmole of hPTH 1-34 in 10
mM ammonium acetate buffer (pH 3.4). The hPTH was
digested for 8 minutes at 45°C before stopping the
reaction by adding ACCA matrix. Time-of-Flight mass

spectrometry was then performed. The resulting
spectrum shown in Fig. 20 includes ion signals for the
pepsin-generated peptides corresponding to amino acids
4-18, 9-24, 8-24, and 18-34.

5   Example 12
                      Sequential Treatment of a
                Biomolecule By Two Different Complexes
        The combination of two different surface bound
complexes was used to modify a biomolecule in sequence
10   as follows. First hPTH was digested for 10 minutes at
35°C, pH 8.1 using an immobilized Au/α-chymotrypsin
sample presentation device as described in Example 2.
A portion of the digest mixture was then transferred to
a cpp activated apparatus prepared as described in
15   Example 2 and the pH of the solution was adjusted to
approximately 5. The cpp digestion was allowed to
proceed for 10 minutes at 35°C before termination by
the addition of the ACCA matrix.
        The mass spectrum of the results of the initial
20   Au/α-chymotrypsin digestion is shown in Fig. 21. Ion
signals resulting from the α-chymotrypsin cleavage of
hPTH are observed. The mass spectrum of the results of
the Au/cpp digestion of the α-chymotrypsin digestion
mixture is shown in Fig. 22. New ion signals indicated
25   by an asterisk "*" are observed. These new signals
correspond to the
C-terminal digestion of the original peptide species.

                            Example 13
                    Purification of Biomolecules
30   The invention can be used to purify biomolecules
as follows. For many biological studies, it is
essential to remove undesirable proteases from
biological solutions. Aprotinin is a single-chain
basic 58-amino-acid polypeptide with a molecular mass

of 6512 Daltons that has a polyvalent inhibitory action
on proteases. Specifically, it inhibits a variety of
enzymes belonging to the family of serine proteases by
binding to the active site of the enzyme, forming tight

5   complexes. A mass spectrometry sample presentation
apparatus is prepared by tethering aprotinin to the
surface in a manner similar to that described in
Example 2. A solution of a polypeptide and a protease
is added to the surface. The protease is removed from

10 solution by the aprotinin. Mass spectrometry is
performed and only the purified polypeptide should be
observed.

Example 14

Preparation of A Sample Presentation Apparatus
15 ### Which Purifies Biomolecules By Ion Exchange
An ion exchange functionality such as a
diethylaminoethyl derivative, a t-butyl amino
derivative (for anion exchange), a sulfite derivative,
a carboxylic acid derivative (for cation exchange), or

20 a combination of the above is immobilized to a sample
presentation surface prepared according to Example 1.

Example 15

Purification of Biomolecules
By Ion Exchange
25 A biomolecule-containing solution is added to a
surface prepared according to Example 14. The ion
exchange functionality bound to the sample presentation
surface exchanges ions with detrimental ionic species
present as impurities in the biomolecule-containing

30 solution.

Example 16
Analysis of DNA Using
Sample Presentation Apparatus

First, the nuclease, snake-venom
5   phosphodiesterase, is attached to the sample
     presentation apparatus surface. This attachment may be
     accomplished using a tethering molecule in a manner
     similar to that described in Example 2. Second, DNA is
     added to the surface. After the phosphodiesterase has
10   produced DNA fragments, a MALDI matrix is added, and
     MALDI time-of-flight mass spectrometry is performed.

Example 17
Analysis of Carbohydrate Using
Sample Presentation Apparatus

15   An enzyme such as mannase which digests
     carbohydrates is attached to the sample presentation
     apparatus surface. This attachment may be accomplished
     using a tethering molecule in a manner similar to that
     described in Example 2. Next, a carbohydrate is added
20   to the surface. After the mannase has produced
     carbohydrate fragments, a MALDI matrix is added, and
     MALDI time-of-flight mass spectrometry is performed.

         Many other variations and modifications of the
     invention will be apparent to those skilled in the art
25   without departing from the spirit and scope of the
     invention. The above-described embodiments are,
     therefore intended to be merely exemplary, and all such
     variations and modifications are intended to be
     included within the scope of the claims.

Claims

1   1.   A sample presentation apparatus comprising:
2   (a) a mass spectrometry sample
3   presentation surface; and
4   (b) a complex bound to the surface, the
5   complex including at least one molecule which
6   chemically modifies a biomolecule.

1   2.   The sample presentation apparatus according to
2   claim 1, wherein the biomolecule comprises at
3   least one selected from the group consisting of
4   polypeptides, DNA, carbohydrates, RNA and
5   combinations thereof.

1   3.   A sample presentation apparatus according to
2   claim 2, wherein the complex comprises a tethering
3   molecule which is attached to the surface, and a
4   reactive molecule which chemically modifies a
5   biomolecule; wherein the tethering molecule is
6   attached to the reactive molecule.

1   4.   A sample presentation apparatus according to
2   claim 2, wherein the complex purifies the
3   biomolecule.

1   5.   A sample presentation apparatus according to
2   claim 2, wherein the complex selectively digests
3   the biomolecule at specific locations.

1   6.   A sample presentation apparatus according to
2   claim 2, wherein the biomolecule is a polypeptide,
3   and the complex comprises a proteolytic enzyme.

1   7.   A sample presentation apparatus according to
2   claim 6, wherein the proteolytic enzyme is

3   selected from the group consisting of trypsin, α-
4   chymotrypsin, pepsin and carboxypeptidase-P.

1   8. A method of making a sample presentation
2   apparatus, which comprises providing a mass
3   spectrometry sample presentation apparatus
4   surface; and binding a complex to the surface
5   wherein the complex includes at least one molecule
6   which chemically modifies a biomolecule.

1   9. A method of making a sample presentation apparatus
2   according to claim 8, wherein the step of binding
3   a complex to the sample presentation surface
4   comprises:
5   (a)   binding at least one end of a
6   tethering molecule to the mass spectrometry sample
7   presentation surface; and
8   (b)   binding a reactive molecule to the
9   tethering molecule wherein the reactive molecule
10   chemically modifies a biomolecule.

1   10. A method of analyzing a biomolecule comprising the
2   steps of:
3   (a)   providing a mass spectrometry
4   presentation surface;
5   (b)   binding at least one complex
6   molecule to the surface, the complex including at
7   least one molecule which chemically modifies a
8   biomolecule;
9   (c)   contacting the biomolecule with the
10   surface, thereby chemically modifying the
11   biomolecule;
12   (d)   applying a MALDI matrix to the
13   surface; and

14   (e)   determining the molecular weight of
15   the chemically modified biomolecule in a mass
16   spectrometer.

1   11.   A method of analyzing a biomolecule according to
2   claim 10, wherein the biomolecule comprises at
3   least one selected from the group consisting of
4   polypeptides, DNA, carbohydrates, RNA and
5   combinations thereof.

1   12.   A method of analyzing a biomolecule according to
2   claim 11, wherein the complex comprises a
3   tethering molecule which is attached to the
4   surface at at least one end of the tethering
5   molecule and a reactive molecule which chemically
6   modifies a biomolecule, wherein the tethering
7   molecule is attached to the reactive molecule.

1   13.   A method of analyzing a biomolecule according to
2   claim 11, wherein the complex purifies a
3   biomolecule.

1   14.   A method of analyzing a biomolecule according to
2   claim 11, wherein the complex selectively digests
3   the biomolecule at specific locations.

1   15.   A method of analyzing a biomolecule according to
2   claim 11, wherein the biomolecule is a polypeptide
3   and the complex comprises a proteolytic enzyme.

1   16.   A method of analyzing a polypeptide according to
2   claim 15, wherein the proteolytic enzyme is
3   selected from the group consisting of trypsin, α-
4   chymotrypsin, pepsin and carboxypeptidase-P.

1   17. A method of analyzing a polypeptide according to
2   claim 15 wherein a reducing agent which cleaves
3   disulfide bonds is added to the surface.

1   18. A method of analyzing a polypeptide according to
2   claim 15 wherein a reducing agent which cleaves
3   disulfide bonds is attached to the surface.

1   19. A method of analyzing a biomolecule according to
2   claim 11, wherein after the step of contacting the
3   biomolecule with the surface, the surface and the
4   biomolecule are incubated in a moist atmosphere.

1   20. A method of analyzing a biomolecule according to
2   claim 11, wherein after the biomolecule is
3   modified by the complex, the biomolecule is
4   exposed to a second reagent molecule which
5   modifies the biomolecule a second time.

1   21. A method of analyzing a biomolecule according to
2   claim 20, wherein the second reagent is different
3   from the reagent molecule in the complex.

1   22. A method of analyzing a biomolecule according to
2   claim 21, wherein the second reagent is a complex
3   which is bound to a surface.

1   23. A method of analyzing a biomolecule according to
2   claim 20, wherein the molecular weight data
3   obtained from analyzing the biomolecules modified
4   by the complex, and the molecular weight data
5   obtained from the biomolecules modified by both
6   the complex and the second reagent, is compared to
7   determine the partial structure of the
8   biomolecule.

1   24. A method of analyzing a biomolecule according to
2   claim 23, wherein the molecular weight data is
3   used to determine a limited amino acid sequence of
4   a peptide.

1   25. A method according to claim 24 further comprising
2   determining at least one nucleic acid sequence
3   corresponding to the limited amino acid sequence,
4   and searching a database containing nucleic acid
5   sequences for the nucleic acid sequence.

1   26. A method of analyzing a biomolecule according to
2   claim 11, wherein the mass spectrometer comprises:
3   (a) a mass spectrometry sample
4   presentation apparatus which comprises said mass
5   spectrometry presentation surface:
6   (b) means for introducing the sample
7   presentation surface into a volatilization
8   chamber;
9   (c) means for volatilizing and ionizing
10   the chemically modified biomolecule; and
11   (d) means for determining the molecular
12   weight to charge ratio of the chemically modified
13   biomolecule ion based on its trajectory in the
14   mass spectrometer.

1   27. A method of analyzing a biomolecule according to
2   claim 26, wherein the mass spectrometer further
3   comprises means for generating an electric field,
4   the field being oriented to cause the chemically
5   modified biomolecule ions to a travel away from
6   the sample presentation apparatus and toward a
7   detecting means.

1   28. A method of analyzing a biomolecule according to
2   claim 26, wherein the means for volatilizing and

3   ionizing the chemically modified biomolecule is a
4   LASER.

1   29. A method of analyzing a biomolecule according to
2   claim 26, wherein the means for determining the
3   molecular weight of the chemically modified
4   biomolecule measures the time of flight for the
5   chemically modified biomolecule.

1   30. A mass spectrometer, which comprises:
2   (a) a mass spectrometry sample
3   presentation apparatus which comprises:
4   (i) a mass spectrometry sample
5   presentation surface; and
6   (ii) a complex bound to the
7   surface, the complex including at least one molecule
8   which chemically modifies a biomolecule;
9   (b) means for introducing the sample
10   presentation surface into a volatilization
11   chamber;
12   (c) means for volatilizing and ionizing
13   the chemically modified biomolecule; and
14   (d) means for determining the molecular
15   weight to charge ratio of the chemically modified
16   biomolecule ion based on its trajectory.

1   31. A mass spectrometer according to claim 30, wherein
2   the mass spectrometer further comprises means for
3   generating an electric field, the field being
4   oriented to cause the chemically modified
5   biomolecule ions to a travel away from the sample
6   presentation apparatus and toward a detecting
7   means.

1   32. A mass spectrometer according to claim 30, wherein
2   the biomolecule comprises at least one selected

3   from the group consisting of polypeptides, DNA,
4   carbohydrates, RNA and combinations thereof.

1   33. A mass spectrometer according to claim 32, wherein
2   the complex comprises a tethering molecule which
3   is attached at at least one end to the surface,
4   and a reactive molecule which chemically modifies
5   a biomolecule wherein the tethering molecule is
6   attached to the reactive molecule.

1   34. A mass spectrometer according to claim 32, wherein
2   the complex purifies a biomolecule.

1   35. A mass spectrometer according to claim 32, wherein
2   the complex selectively digests the biomolecule at
3   specific locations in the biomolecule.

1   36. A mass spectrometer according to claim 32, wherein
2   the biomolecule is a polypeptide, and the reactive
3   molecule is a proteolytic enzyme.

1   37. A mass spectrometer according to claim 36, wherein
2   the proteolytic enzyme is selected from the group
3   consisting of trypsin, α-chymotrypsin, pepsin and
4   carboxypeptidase-P.

1   38. A mass spectrometer according to claim 30, wherein
2   the means for volatilizing and ionizing the
3   chemically modified biomolecule is a LASER.

1   39. A mass spectrometer according to claim 38, wherein
2   the means for determining the molecular weight of
3   the chemically modified biomolecule measures the
4   time of flight for the chemically modified
5   biomolecule.

1 40. A method of analyzing a polypeptide according to
2   claim 15, wherein after the molecular weight of a
3   polypeptide fragment is determined, the molecular
4   weight is subtracted from the weight of a next
5   lower molecular weight polypeptide fragment to
6   determine which amino acid is lacking from the
7   lower molecular weight polypeptide fragment.

1 41. A method of analyzing a polypeptide according to
2   claim 40, wherein a limited sequence of a
3   polypeptide, containing amino acids, is determined
4   by sequentially analyzing lower molecular weight
5   polypeptide fragments to determine which amino
6   acids they lack, and further comprising
7   determining at least one nucleic acid sequence
8   corresponding to the limited amino acid sequent
9   and searching a database containing nucleic acid
10   sequences for the nucleic acid sequence.

AMENDED CLAIMS
[received by the International Bureau on 27 November 1996 (27.11.96);
original claims 1-41 replaced by amended claims 1-43 (9 pages)]

Claims

1   1.   A sample presentation apparatus comprising:
2   (a)   a mass spectrometry sample
3   presentation apparatus having a mass spectrometry
4   sample presentation surface; and
5   (b)   a complex immobilized on the
6   surface, the complex including at least one
7   molecule which chemically modifies a biomolecule,
8   wherein the biomolecule is selected from the group
9   consisting of polypeptides, DNA, carbohydrates,
10 RNA, and combinations thereof.

1   2.   A sample presentation apparatus according to claim
2   1, wherein the complex comprises:
3   (a)   a tethering molecule which is
4   attached to the sample presentation surface at at
5   least one end of the tethering molecule, and
6   (b) a reactive molecule which chemically
7   modifies a biomolecule;
8   wherein the tethering molecule is
9   attached to the reactive molecule.

1   4.   A sample presentation apparatus according to
2   claim 1, wherein the complex purifies the
3   biomolecule.

1   5.   A sample presentation apparatus according to
2   claim 1, wherein the complex selectively digests
3   the biomolecule at specific locations.

1   6.   A sample presentation apparatus according to
2   claim 3, wherein the biomolecule is a polypeptide,
3   and the reactive molecule is a proteolytic enzyme.

1   7.   A sample presentation apparatus according to
2   claim 6, wherein the proteolytic enzyme is

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3   selected from the group consisting of trypsin, α-
4   chymotrypsin, pepsin and carboxypeptidase-P.

1   8.   A method of making a sample presentation
2   apparatus, which comprises providing a mass
3   spectrometry sample presentation apparatus having
4   a mass spectrometry sample presentation surface;
5   and binding a complex to the surface wherein the
6   complex includes at least one molecule which
7   chemically modifies a biomolecule, wherein the
8   biomolecule is selected from the group consisting
9   of polypeptides, DNA, carbohydrates, RNA, and
10   combinations thereof.

1   9.   A method of making a sample presentation apparatus
2   according to claim 8, wherein the step of binding
3   a complex to the sample presentation surface
4   comprises:
5   (a)   binding at least one end of a
6   tethering molecule to the mass spectrometry sample
7   presentation surface; and
8   (b)   binding a reactive molecule to the
9   tethering molecule wherein the reactive molecule
10   chemically modifies a biomolecule, wherein the
11   biomolecule is selected from the group consisting
12   of polypeptides, DNA, carbohydrates, RNA, and
13   combinations thereof.

1   10.   A method of analyzing a biomolecule comprising the
2   steps of:
3   (a)   providing a mass spectrometry
4   sample presentation surface;
5   (b)   contacting at least one complex
6   including at least one molecule which chemically
7   modifies a biomolecule with the sample

8   presentation surface to form a surface-bound
9   complex;
10   (c)   removing any unbound complex from
11   the sample presentation surface;
12   (d)   contacting the biomolecule with the
13   surface-bound complex, thereby chemically
14   modifying the biomolecule;
15   (e)   applying a MALDI matrix to the
16   sample presentation surface; and
17   (f)   determining the molecular weight of
18   the chemically modified biomolecule in a mass
19   spectrometer.

1   11.   A method of analyzing a biomolecule according to
2   claim 10, wherein the biomolecule comprises at
3   least one selected from the group consisting of
4   polypeptides, DNA, carbohydrates, RNA and
5   combinations thereof.

1   12.   A method of analyzing a biomolecule according to
2   claim 11, wherein the complex comprises:
3   (a)   a tethering molecule which is
4   attached to the sample presentation surface, at at
5   least one end of the tethering molecule, and
6   (b)   a reactive molecule which
7   chemically modifies a biomolecule,
8   wherein the tethering molecule is also
9   attached to the reactive molecule.

1   13.   A method of analyzing a biomolecule according to
2   claim 11, wherein the complex purifies a
3   biomolecule.

1   14.   A method of analyzing a biomolecule according to
2   claim 11, wherein the complex sel ctively digests
3   the biom l cule at specific locations.

1   15. A method of analyzing a biomolecule according to
2   claim 11, wherein the biomolecule is a polypeptide
3   and the complex comprises a proteolytic enzyme.

1   16. A method of analyzing a polypeptide according to
2   claim 15, wherein the proteolytic enzyme is
3   selected from the group consisting of trypsin, α-
4   chymotrypsin, pepsin and carboxypeptidase-P.

1   17. A method of analyzing a polypeptide according to
2   claim 15 wherein a reducing agent which cleaves
3   disulfide bonds is added to the surface.

1   18. A method of analyzing a polypeptide according to
2   claim 15 wherein a reducing agent which cleaves
3   disulfide bonds is attached to the surface.

1   19. A method of analyzing a biomolecule according to
2   claim 11, wherein after the step of contacting the
3   biomolecule with the surface-bound complex, the
4   surface-bound complex and the biomolecule are
5   incubated in a moist atmosphere.

1   20. A method of analyzing a biomolecule according to
2   claim 11, wherein after the biomolecule is
3   modified by the complex, the biomolecule is
4   exposed to a second reagent molecule which
5   modifies the biomolecule a second time.

1   21. A method of analyzing a biomolecule according to
2   claim 20, wherein the second reagent molecule is
3   different from the reactive molecule.

1   22. A method of analyzing a biomolecule according to
2   claim 21, wherein the second reagent molecule is a
3   complex which is bound to a surface.

1   23. A method of analyzing a biomolecule according to
2   claim 20, wherein the molecular weight data
3   obtained from analyzing the biomolecules modified
4   by the complex, and the molecular weight data
5   obtained from the biomolecules modified by both
6   the complex and the second reagent molecule, is
7   compared to determine the partial structure of the
8   biomolecule.

1   24. A method of analyzing a biomolecule according to
2   claim 23, wherein the molecular weight data is
3   used to determine a limited amino acid sequence of
4   a peptide.

1   25. A method according to claim 24 further comprising
2   determining at least one nucleic acid sequence
3   corresponding to the limited amino acid sequence,
4   and searching a database containing nucleic acid
5   sequences for the nucleic acid sequence.

1   26. A method of analyzing a biomolecule according to
2   claim 11, wherein the mass spectrometer comprises:
3   (a)   a mass spectrometry sample
4   presentation apparatus which comprises said mass
5   spectrometry presentation surface:
6   (b)   means for introducing the sample
7   presentation surface into a volatilization
8   chamber;
9   (c)   means for volatilizing and ionizing
10   the chemically modified biomolecule; and
11   (d)   means for determining the molecular
12   weight to charge ratio of the chemically modified
13   biomolecule ion based on its trajectory in the
14   mass spectrometer.

1 27. A method of analyzing a biomolecule according to
2   claim 26, wherein the mass spectrometer further
3   comprises means for generating an electric field,
4   the field being oriented to cause the chemically
5   modified biomolecule ions to a travel away from
6   the sample presentation surface and toward a
7   detecting means.

1 28. A method of analyzing a biomolecule according to
2   claim 26, wherein the means for volatilizing and
3   ionizing the chemically modified biomolecule is a
4   LASER.

1 29. A method of analyzing a biomolecule according to
2   claim 26, wherein the means for determining the
3   molecular weight of the chemically modified
4   biomolecule measures the time of flight for the
5   chemically modified biomolecule.

1 31. A mass spectrometer according to claim 43, wherein
2   the mass spectrometer further comprises means for
3   generating an electric field, the field being
4   oriented to cause the chemically modified
5   biomolecule ions to travel away from the sample
6   presentation apparatus and toward a detecting
7   means.

1 32. A mass spectrometer according to claim 43, wherein
2   the biomolecule comprises at least one selected
3   from the group consisting of polypeptides, DNA,
4   carbohydrates, RNA and combinations thereof.

1 33. A mass spectrometer according to claim 32, wherein
2   the complex comprises a tethering molecule which
3   is attached at at least one end to the surface,
4   and a reactive molecule which chemically modifi s

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5   a biomolecule wherein the tethering molecule is
6   attached to the reactive molecule.

1   34. A mass spectrometer according to claim 32, wherein
2   the complex purifies a biomolecule.

1   35. A mass spectrometer according to claim 32, wherein
2   the complex selectively digests the biomolecule at
3   specific locations in the biomolecule.

1   36. A mass spectrometer according to claim 33, wherein
2   the biomolecule is a polypeptide, and the reactive
3   molecule is a proteolytic enzyme.

1   37. A mass spectrometer according to claim 36, wherein
2   the proteolytic enzyme is selected from the group
3   consisting of trypsin, α-chymotrypsin, pepsin and
4   carboxypeptidase-P.

1   38. A mass spectrometer according to claim 43, wherein
2   the means for volatilizing the matrix material is
3   a LASER.

1   39. A mass spectrometer according to claim 38, wherein
2   the means for determining the molecular weight to
3   charge ratio of the chemically modified
4   biomolecule measures the time of flight for the
5   chemically modified biomolecule.

1   40. A method of analyzing a polypeptide according to
2   claim 15, wherein after the molecular weight of a
3   polypeptide fragment is determined, the molecular
4   weight is subtracted from the weight of a next
5   lower molecular weight polypeptide fragment to
6   determine which amino acid is lacking from the
7   lower molecular weight polypeptide fragment.

1   41.   A method of analyzing a polypeptide according to
2   claim 40, wherein a limited sequence of a
3   polypeptide containing amino acids, is determined
4   by sequentially analyzing lower molecular weight
5   polypeptide fragments to determine which amino
6   acids they lack, and further comprising
7   determining at least one nucleic acid sequence
8   corresponding to the limited amino acid sequence
9   and searching a database containing nucleic acid
10   sequences for the nucleic acid sequence.

1   42.   A mass spectrometer, which comprises a mass
2   spectrometry sample presentation apparatus which
3   comprises:
4   (a)   a mass spectrometry sample
5   presentation surface; and
6   (b)   a complex immobilized on the
7   surface, the complex including at least one
8   molecule which chemically modifies a biomolecule
9   wherein the complex is not incorporated in a MALDI
10   matrix and is not volatilized in a mass
11   spectrometer.

1   43.   A mass spectrometer according to claim 42, which
2   further comprises:
3   (a)   means for introducing a sample
4   presentation surface into a volatilization
5   chamber;
6   (b)   means for volatilizing and ionizing
7   a chemically modified biomolecule, thereby forming
8   a chemically modified biomolecule ion, said means
9   for volatilizing comprising (i) a matrix material
10   which solubilizes the chemically modified
11   biomolecule and absorbs light energy at a
12   frequency easily accessible by a laser, wherein
13   the matrix material is applied over the complex,

14   and (ii) means for volatilizing the matrix
15   material; and
16   (c) means for determining a molecular
17   weight to charge ratio of the chemically modified
18   biomolecule ion based on its trajectory.

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FIG. 1
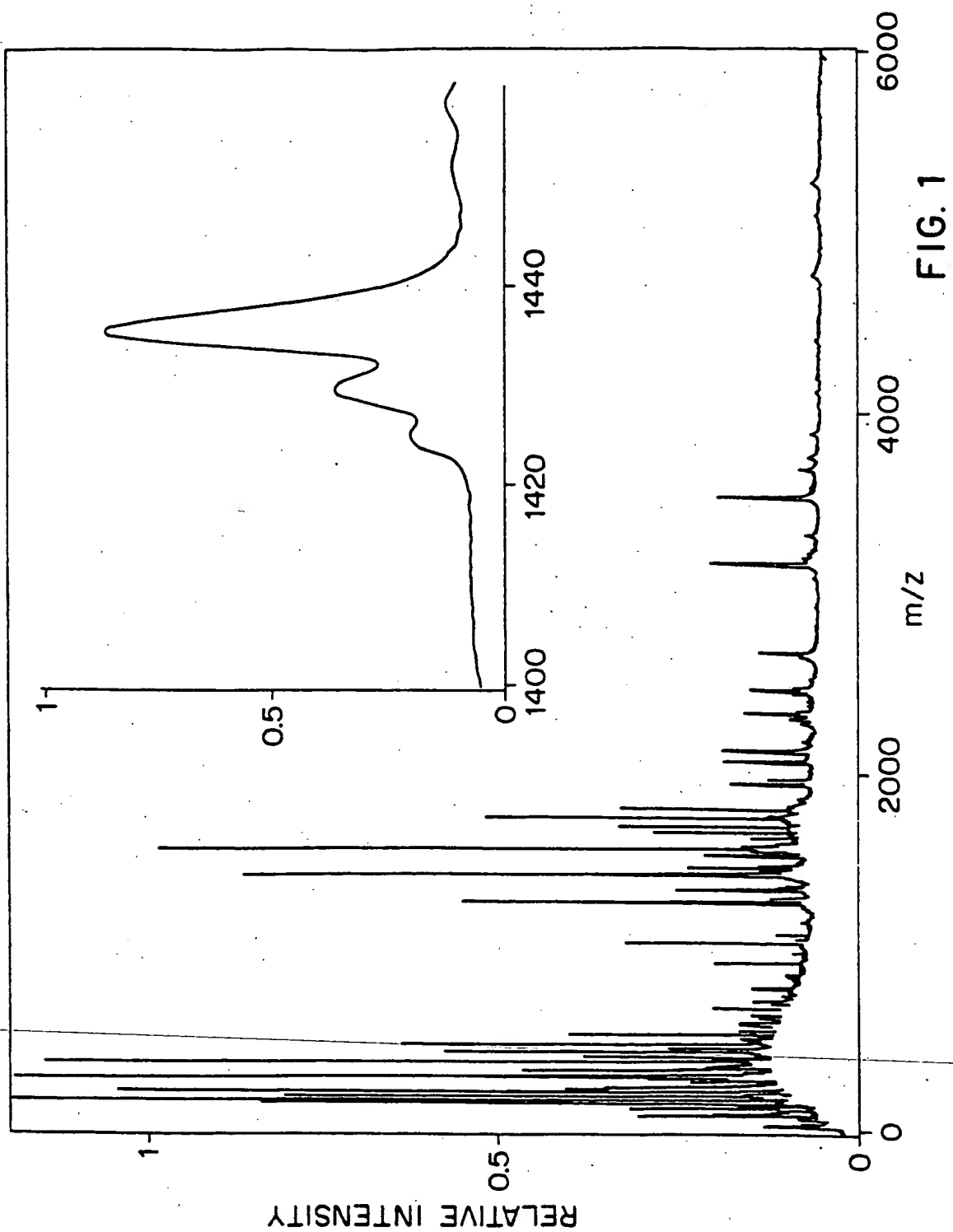
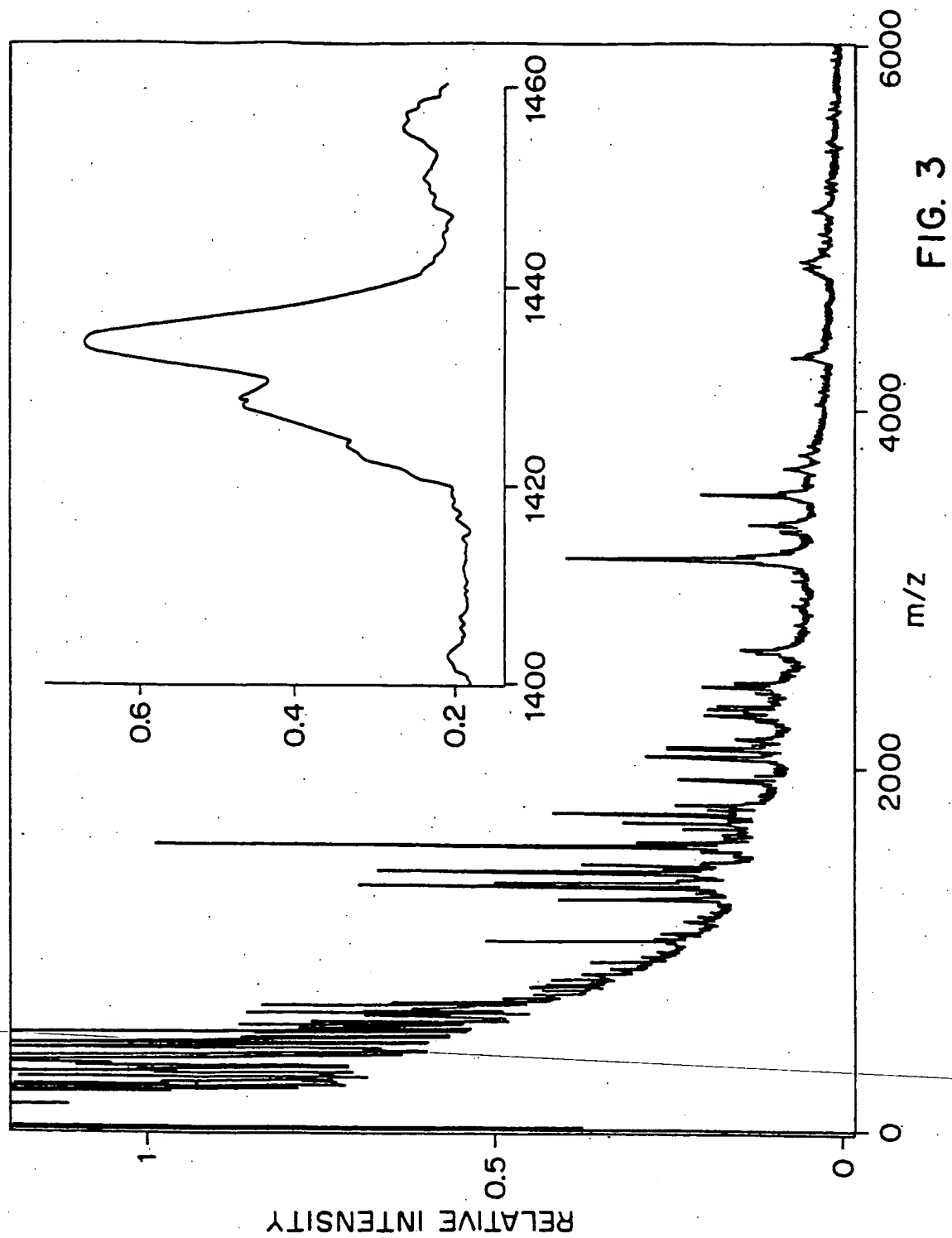RESIDUES OF LYSOZYME FOLLOWING 10 MINUTE DIGEST
ON AN Au/TRYPSIN PROBE TIP

MASS(observed)Da	MASS(calculated)Da	Δ Da	RESIDUES
936.6	937.02	−0.42	62−68
1046	1045.54	0.46	117−125
1277.4	1276.64	0.76	22−33
1424.8	1424.78	0.02	1−13
1429.6	1428.64	0.96	34−45
1435.5	1434.63	0.87	62−73
1475.8	1474.75	1.05	117−129
1548	1546.78	1.22	113−125
1581	1580.96	0.04	1−14
1677.1	1676.90	0.20	98−112
1707.2	1707.04	0.16	115−129
1755	1754.86	0.14	46−61
1805.4	1805.08	0.32	97−112
1947.5	1947.19	0.31	98−114
1977.8	1977.33	0.47	113−129
2076.1	2075.37	0.73	97−114
2126	2125.38	0.62	15−33
2281.7	2281.57	0.13	14−33
2306.9	2304.69	2.21	97−116
2339.2	2336.02	2.48	74−96
2437.1	2436.88	0.22	1−21
2467.4	2464.90	2.50	74−97
2680.8	2679.94	0.86	22−45
3172.4	3171.41	0.99	46.73
3334.8	3333.84	0.96	97−125
3537.2	3535.68	1.52	15−45
3689.9	3692.05	−2.15	14−45
3754.4	3751.26	3.14	62−96
4125.5	4122.78	2.72	74−112
4418.4	4415.79	2.61	74−114
5098	5097.81	0.19	1−45
5273	5271.70	1.30	15−61
5488.3	5487.10	1.20	46−96

FIG. 2

FIG. 3

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FIG. 4

FIG. 5
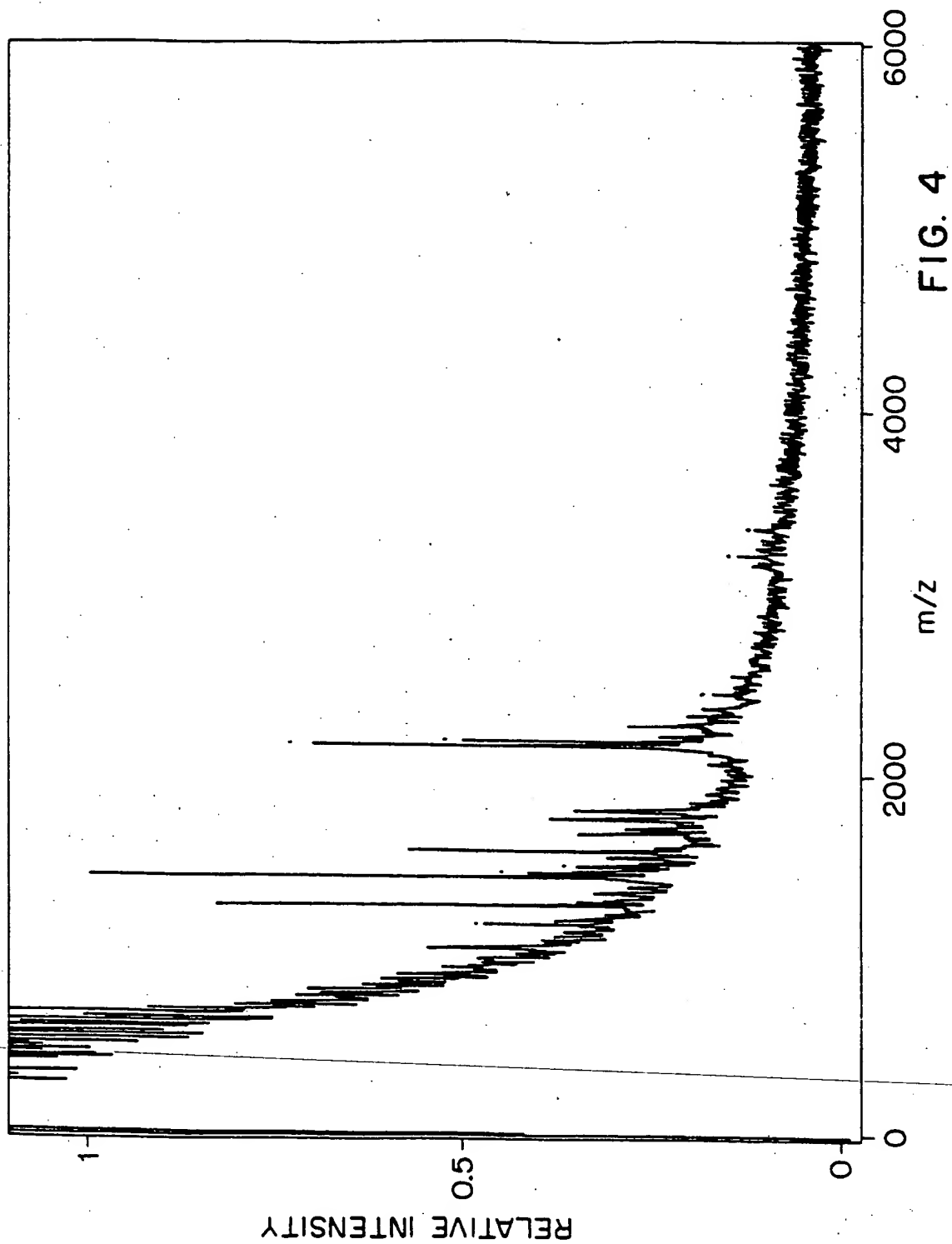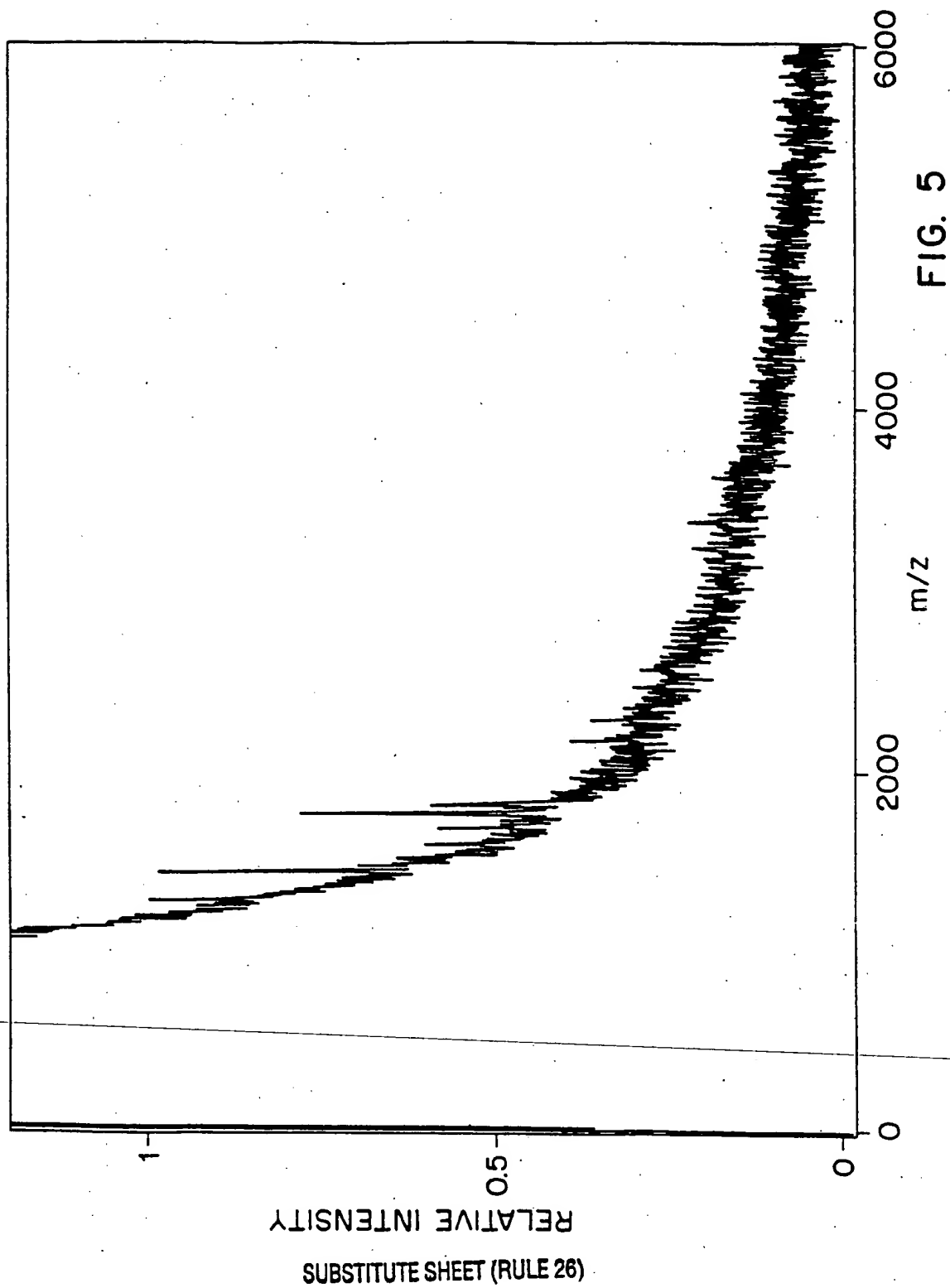
RESIDUES OF LYSOZYME FOLLOWING 10 MINUTE DIGEST OF
1 PMOL WITH FREE–TRYPSIN AND AGAROSE-IMMOBILIZED
TRYPSIN

FREE TRYPSIN MASS(observed)Da	IMMOBILIZED TRYPSIN MASS(observed)Da	RESIDUES	AUTOLYSIS PRODUCT
935.3	936.5	62–68	
1046.1	1046.1	117–125	
1176.7			X
1277.4	1276.9	115–125	
1435.6	1434.9	62–73	
1458.9			X
1476.9		117–129	
1497.9			X
1548.8		13–125	
1582.4	1581.8	1–14	
1678.2	1677.6	98–112	
1709.4		115–129	
1756.7	1755.5	46–61	
1806.4	1805.8	97–112	
2166	2164.4		X
2190.6			X
2274	2274.8		
2471.5			X
3168		46–73	
3227.9			X
3372.5	3371.1		X

FIG. 6
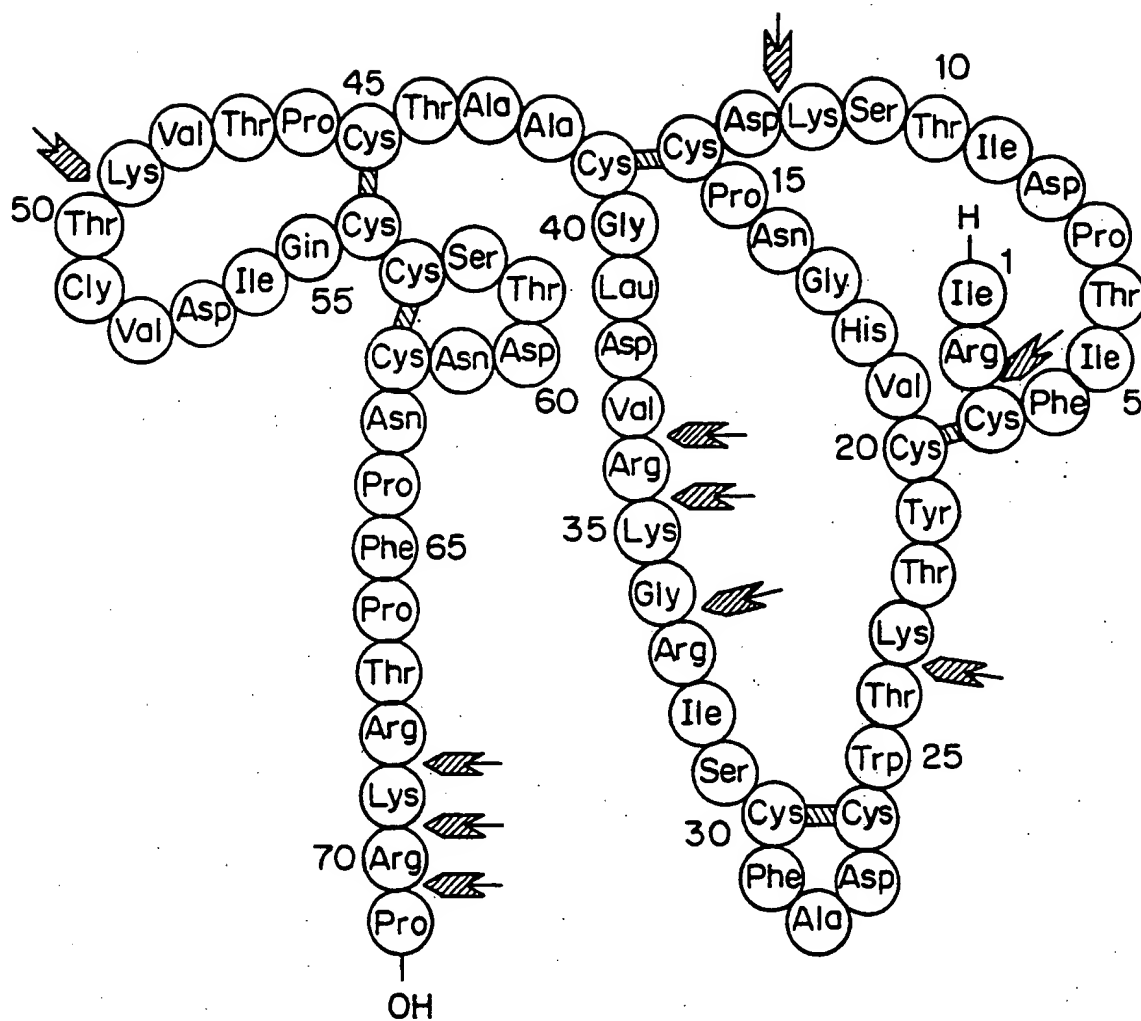
FIG. 7

= TRYPSIN CLEAVAGE SITE

FIG. 8

α-COBRATOXIN-30 MINUTE DIGEST ON "ACTIVE"
Au/TRYPSIN PROBE (NATIVE)

FIG. 9

α–COBRATOXIN – 30 MINUTE DIGEST ON "ACTIVE"
Au/TRYPSIN PROBE (DTT PRESENT)

FIG. 10

α-COBRATOXIN-30 MINUTE DIGEST ON "ACTIVE"
Au/TRYPSIN PROBE (DTT PRESENT)

FIG. 11

RELATIVE INTENSITY

m/z

FIG. 12

HUMAN PARATHYROID HORMONE 1-34
Au/α-CHYMOTRYPSIN: 10 MINUTES: 40 °C: pH 8:1

FIG. 13
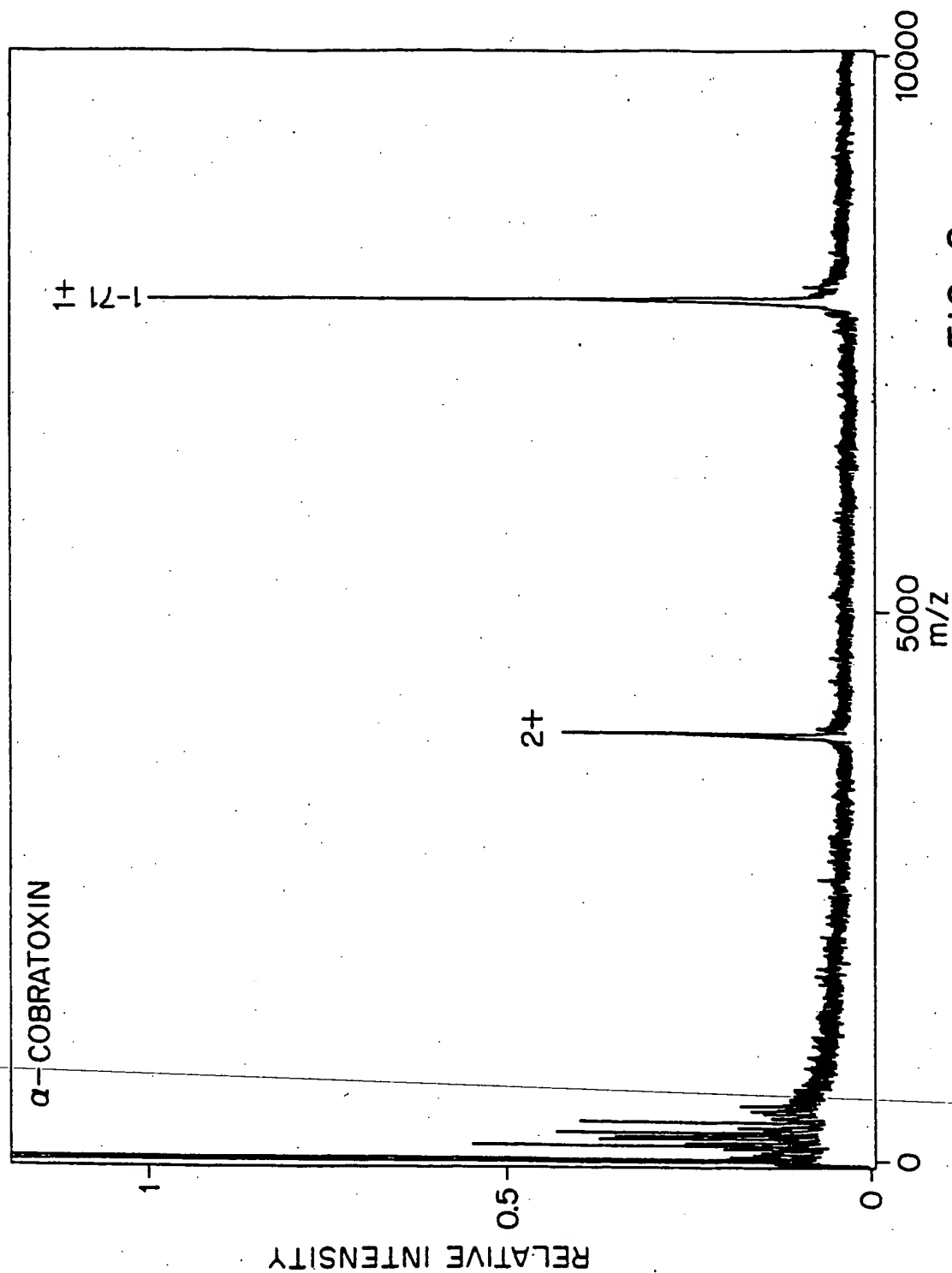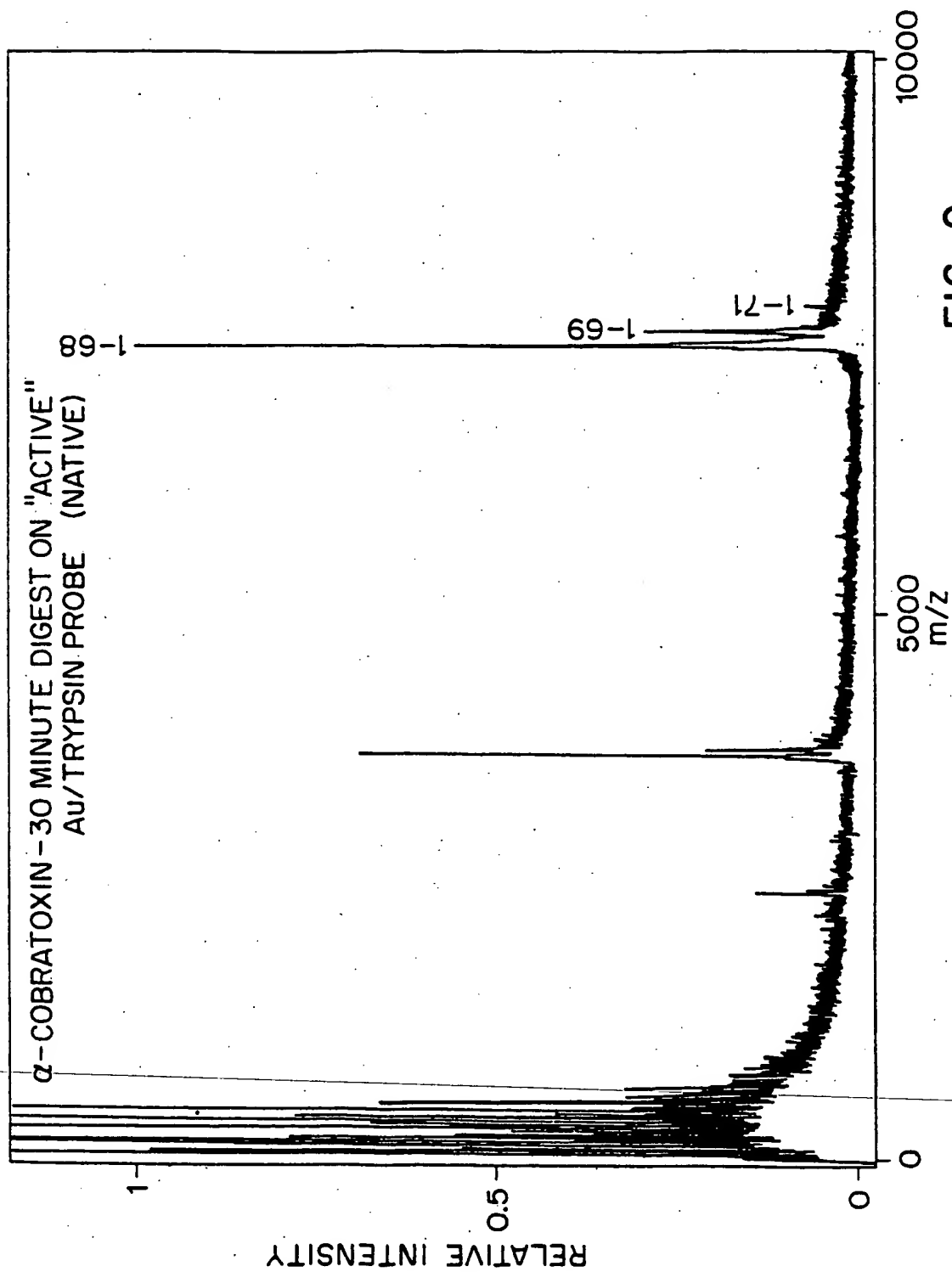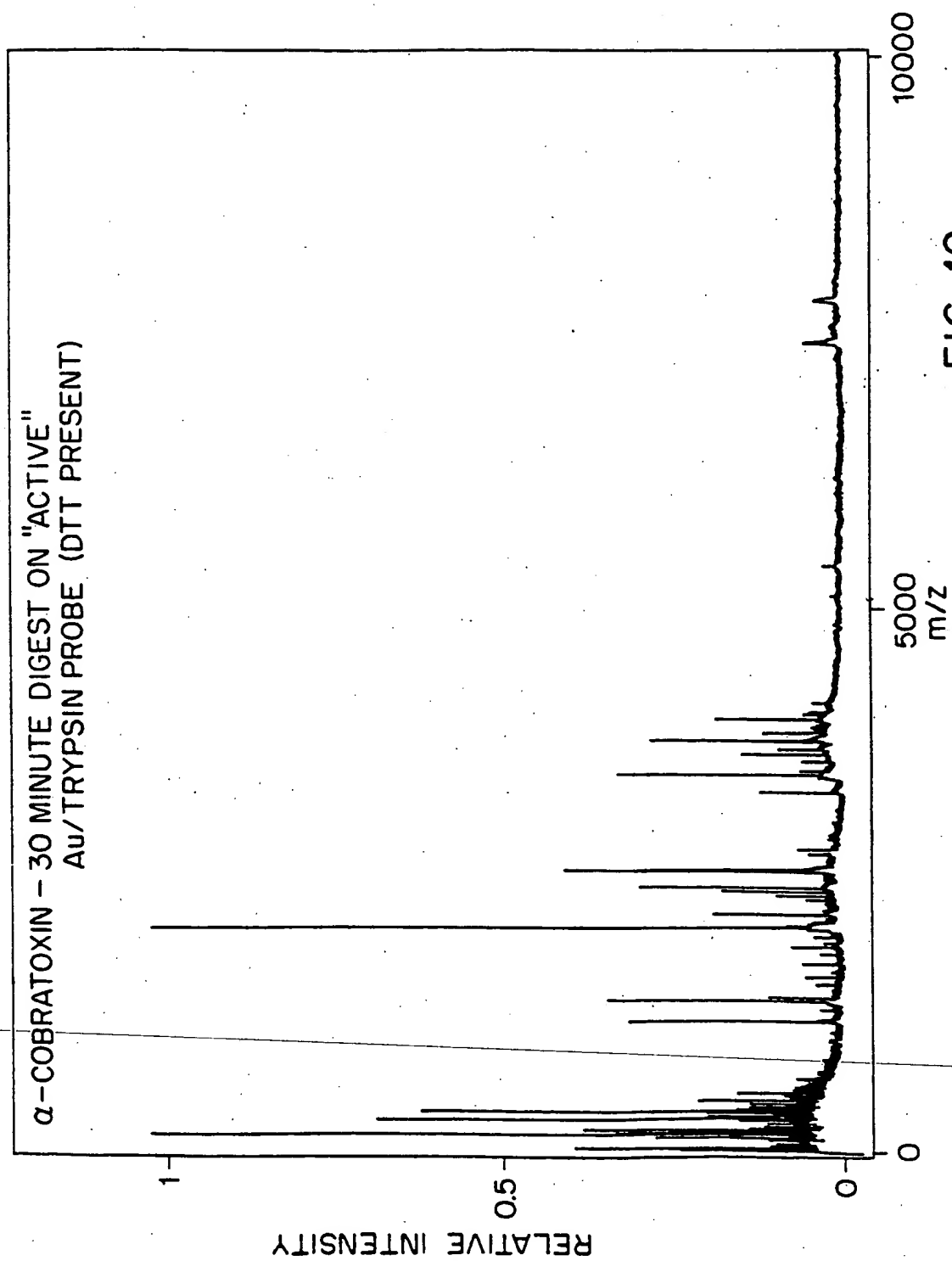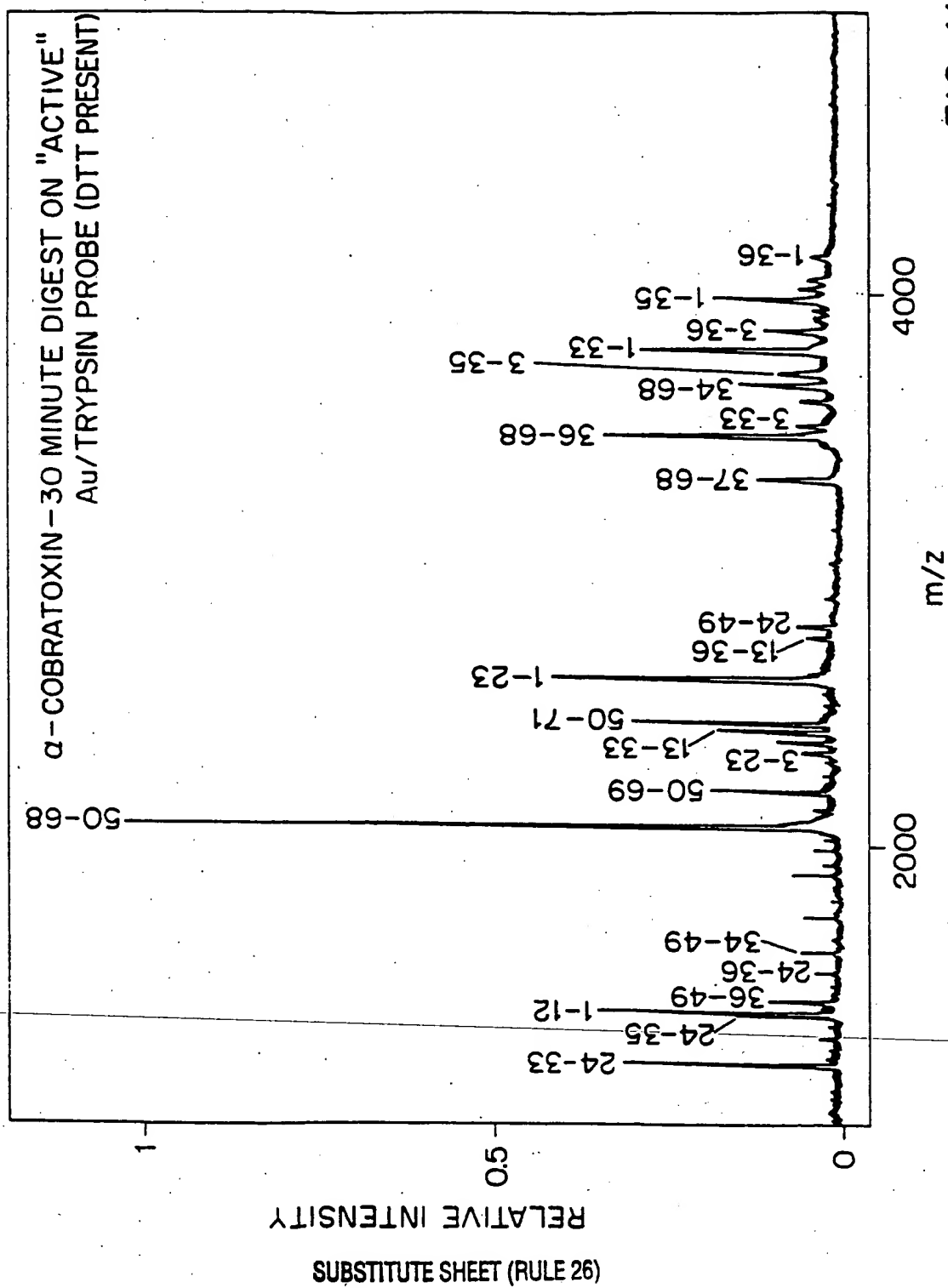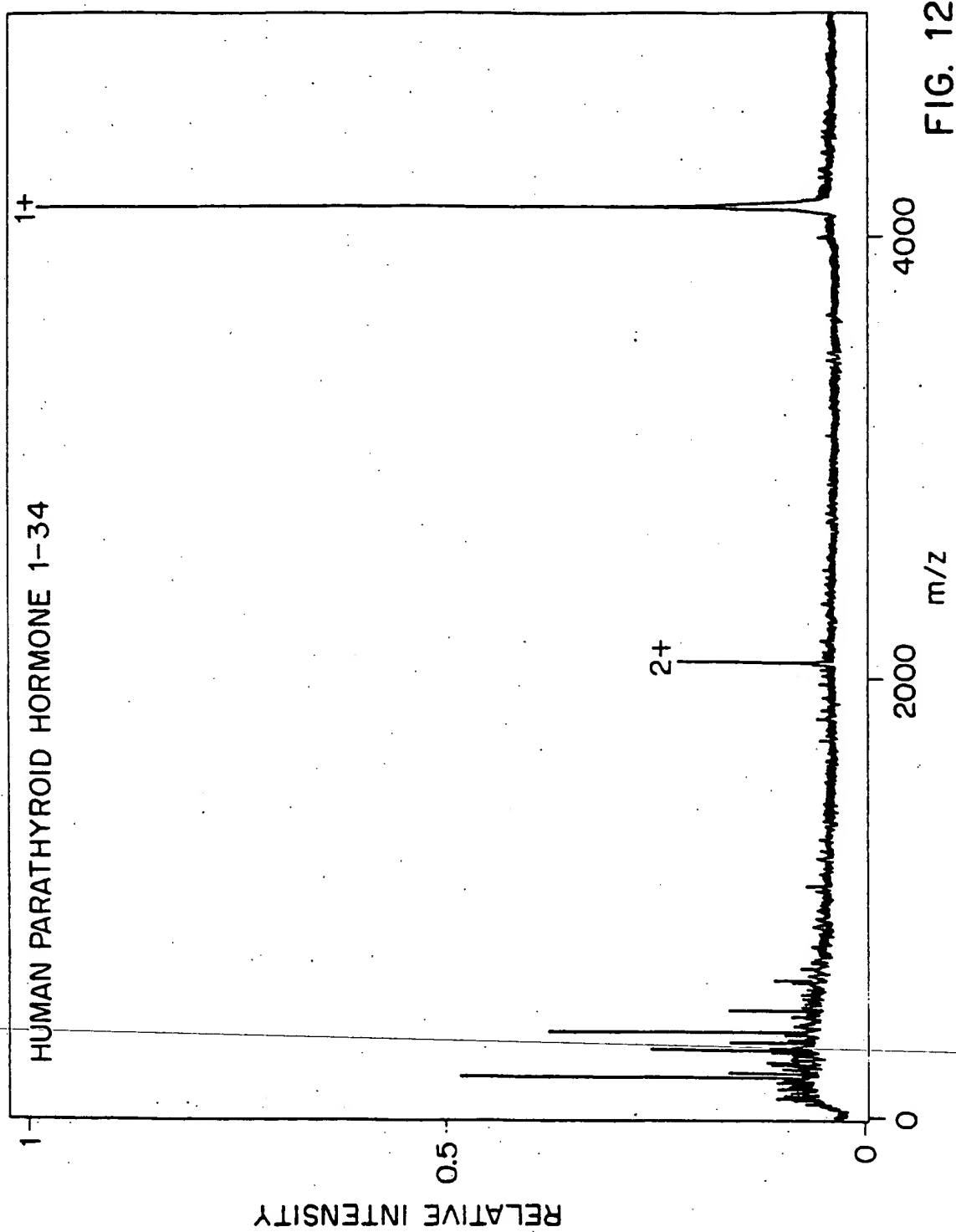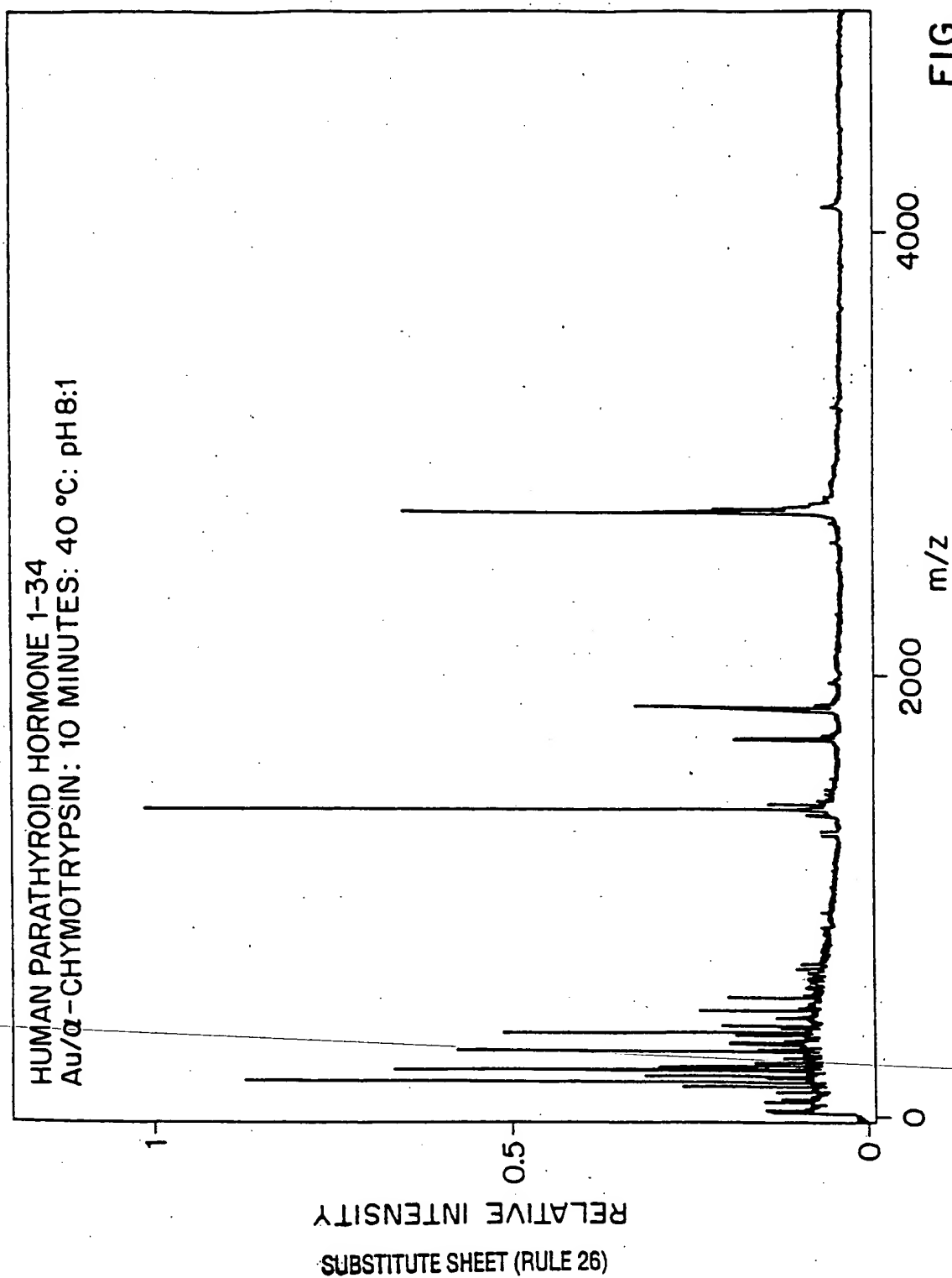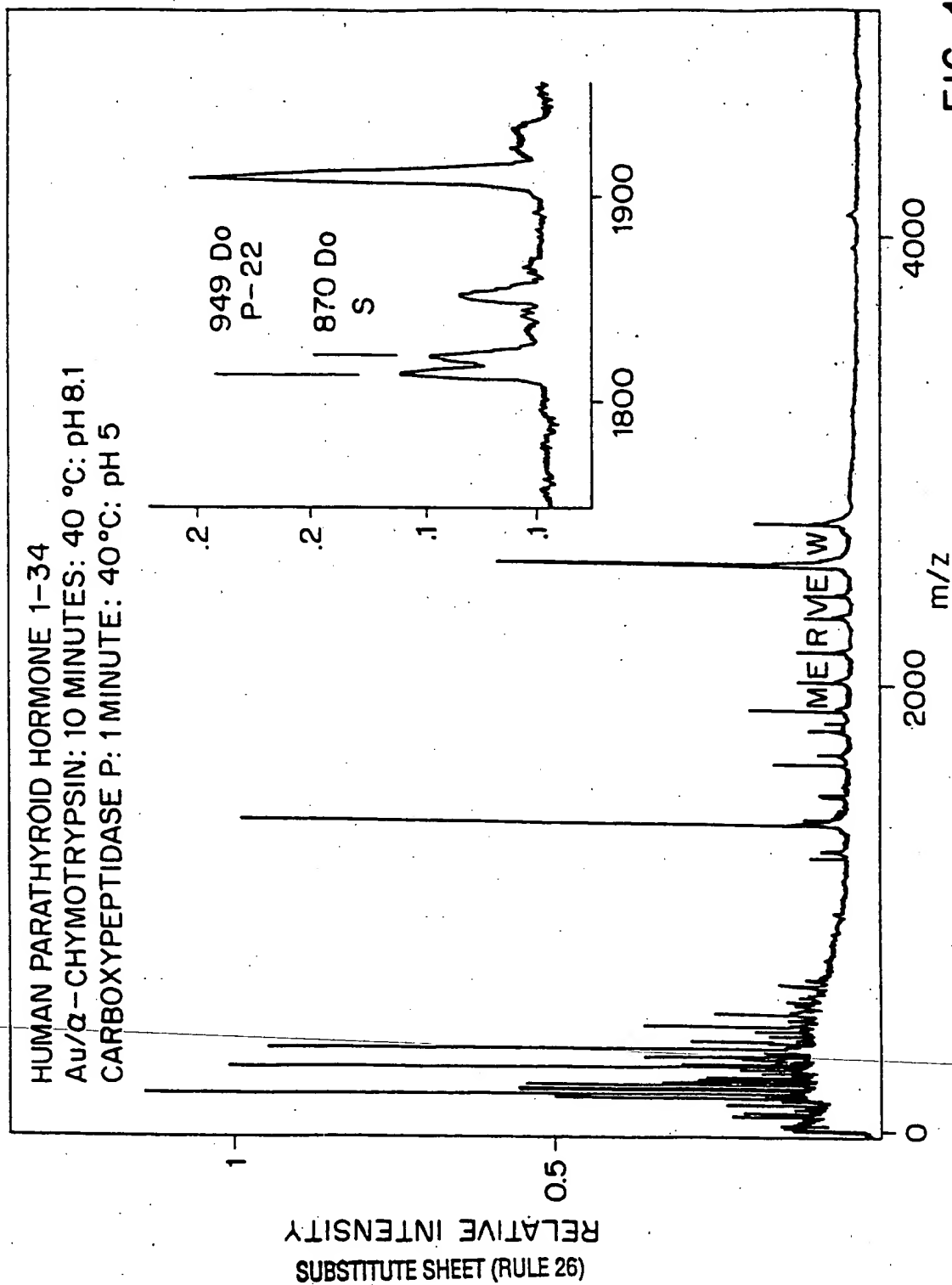
FIG. 14

hPTH Au/α-CHYMOTRYPSIN/CARBOXYPEPTIDASE P

ENDOPEPTIDASE SIGNAL	LADDER SIGNAL	DIFFERENCE	RESIDUE	ERROR
2,738.7	2,738.9	0		
	2,552.4	186.5	W	+0.3
	2,423.5	128.9	E	−0.2
	2,324.3	99.2	V	+0.1
	2,168.1	156.2	R	0.0
	2,038.8	129.3	E	+0.2
	1,907.6	131.2	M	0.0
1,850.0				
	1,820.6	87.0	S	−0.1
1,706.2				
1,398.5				

FIG. 15

HUMAN PARATHROID HORMONE 1-34
Au/TRYPSIN: 5 MINUTES: 45°C: pH 8.1

FIG. 16

FIG. 17
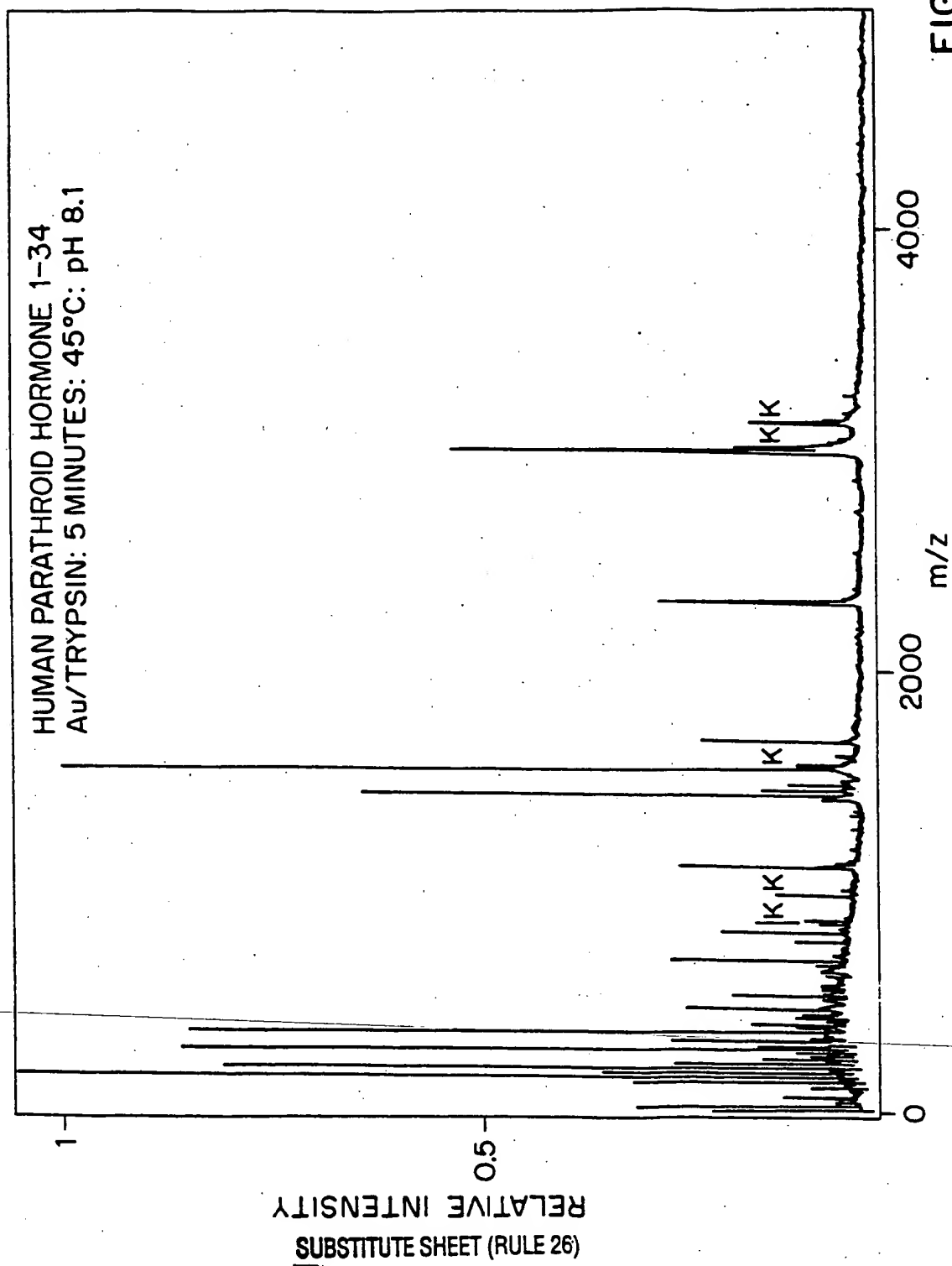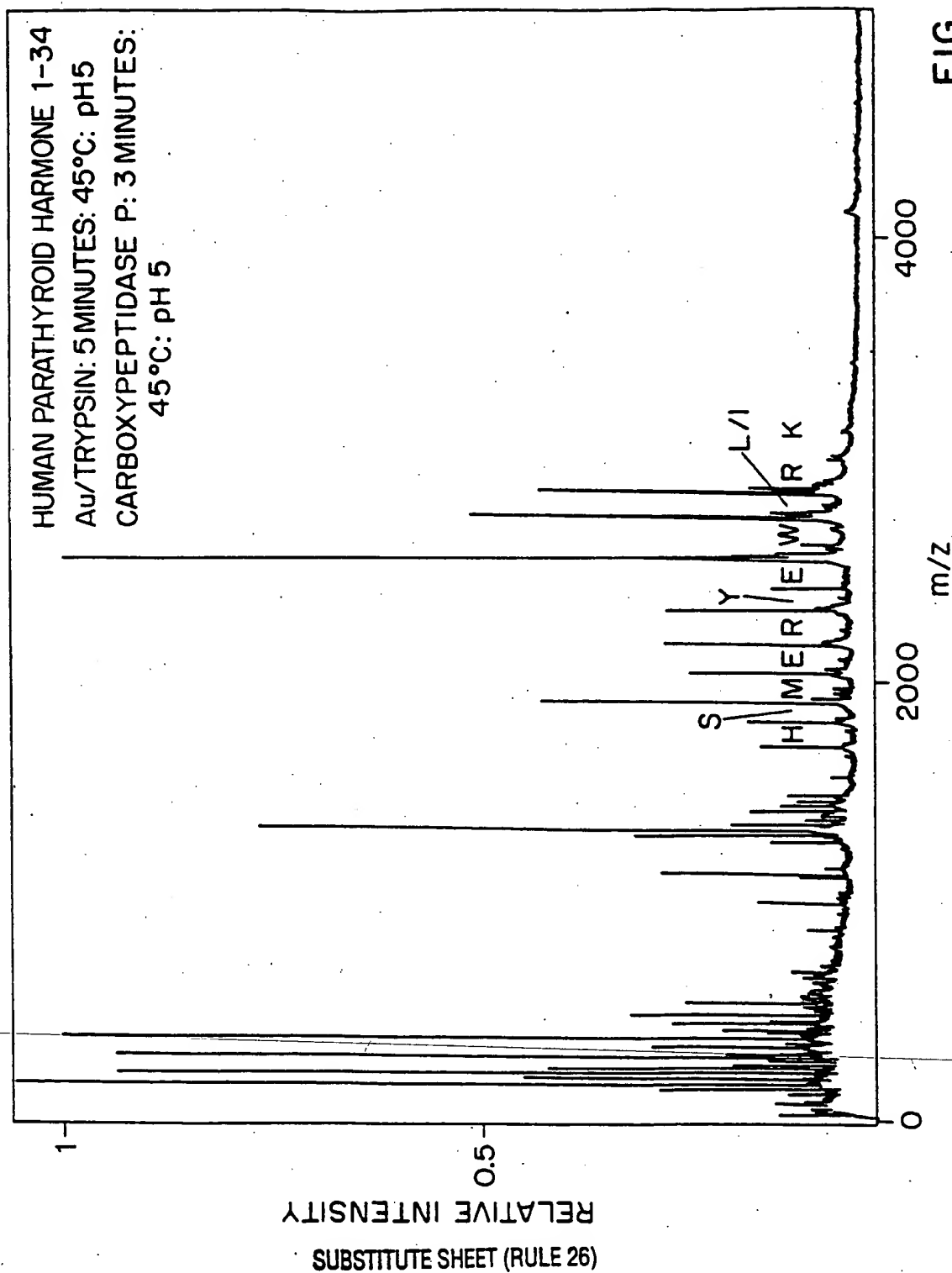
hPTH Au/TRYPSIN/CARBOXYPEPTIDASE P
(*DENOTES LYS-LYS)

ENDOPEPITDASE SIGNAL	LADDER SIGNAL	DIFFERENCE	RESIDUE	ERROR
*3,264.3				
*3,136.1	3,136.1	128.2	K	0.0
*3,007.9	3,007.8	128.3	K	+0.1
	2,851.8	156.0	R	−0.2
	2,738.6	113.2	L/I	0.0
	2,552.4	186.2	W	0.0
	2,423.5	128.9	E	−0.2
2,323.8	2,324.0	99.5	V	+0.4
	2,167.8	156.2	R	0.0
	2,038.5	129.3	E	+0.2
	1,907.4	131.1	M	0.0
	1,820.3	87.1	S	0.0
	1,706.3	114.0	N	−0.1
1,698.2				
1,570.0		128.2	K	0.0
1,455.9				
*1,128.7				
*1,000.5		128.2	K	0.0
886.4				
*872.3		128.2	K	0.0
830.4				
702.3		128.1	K	−0.1

FIG. 18
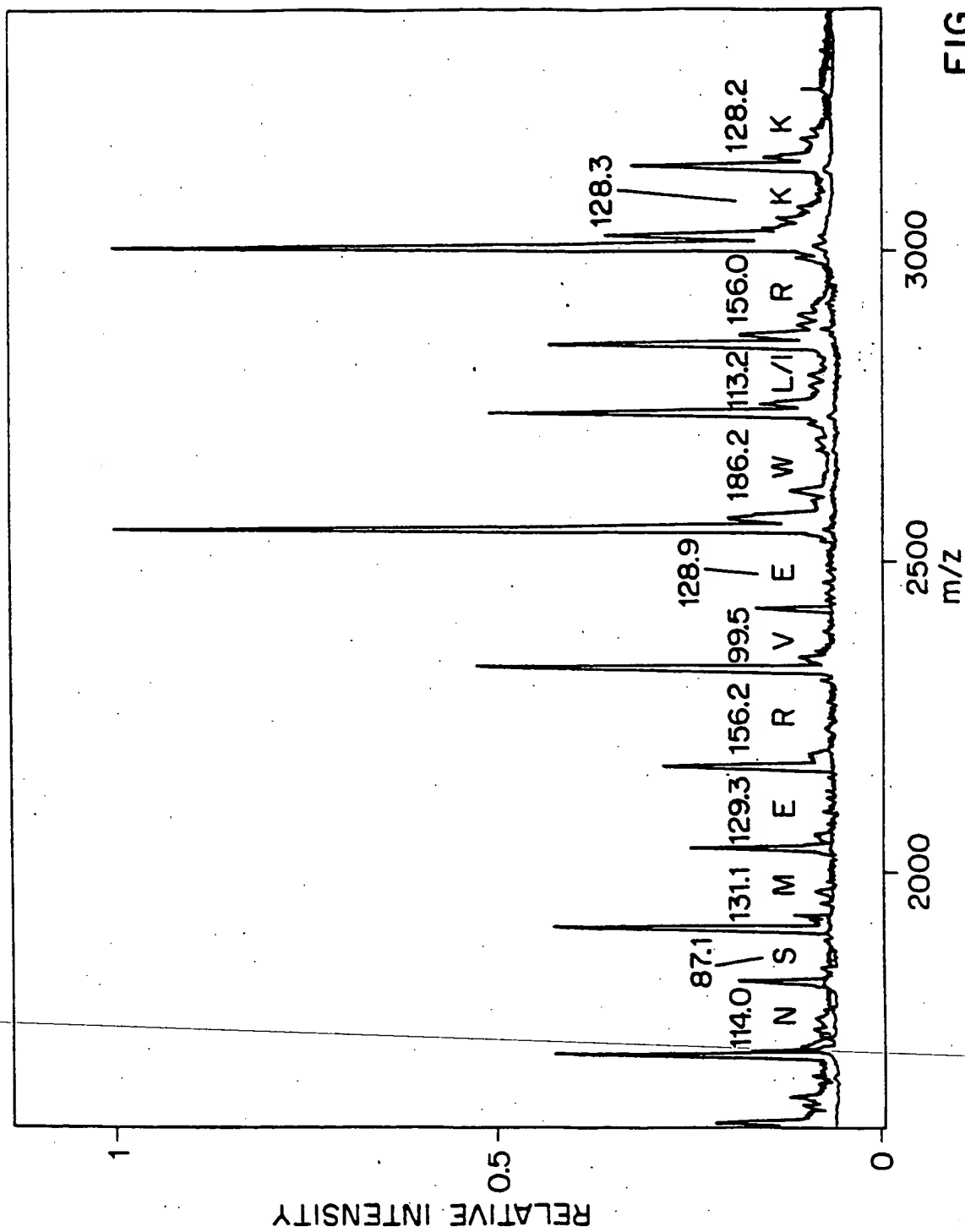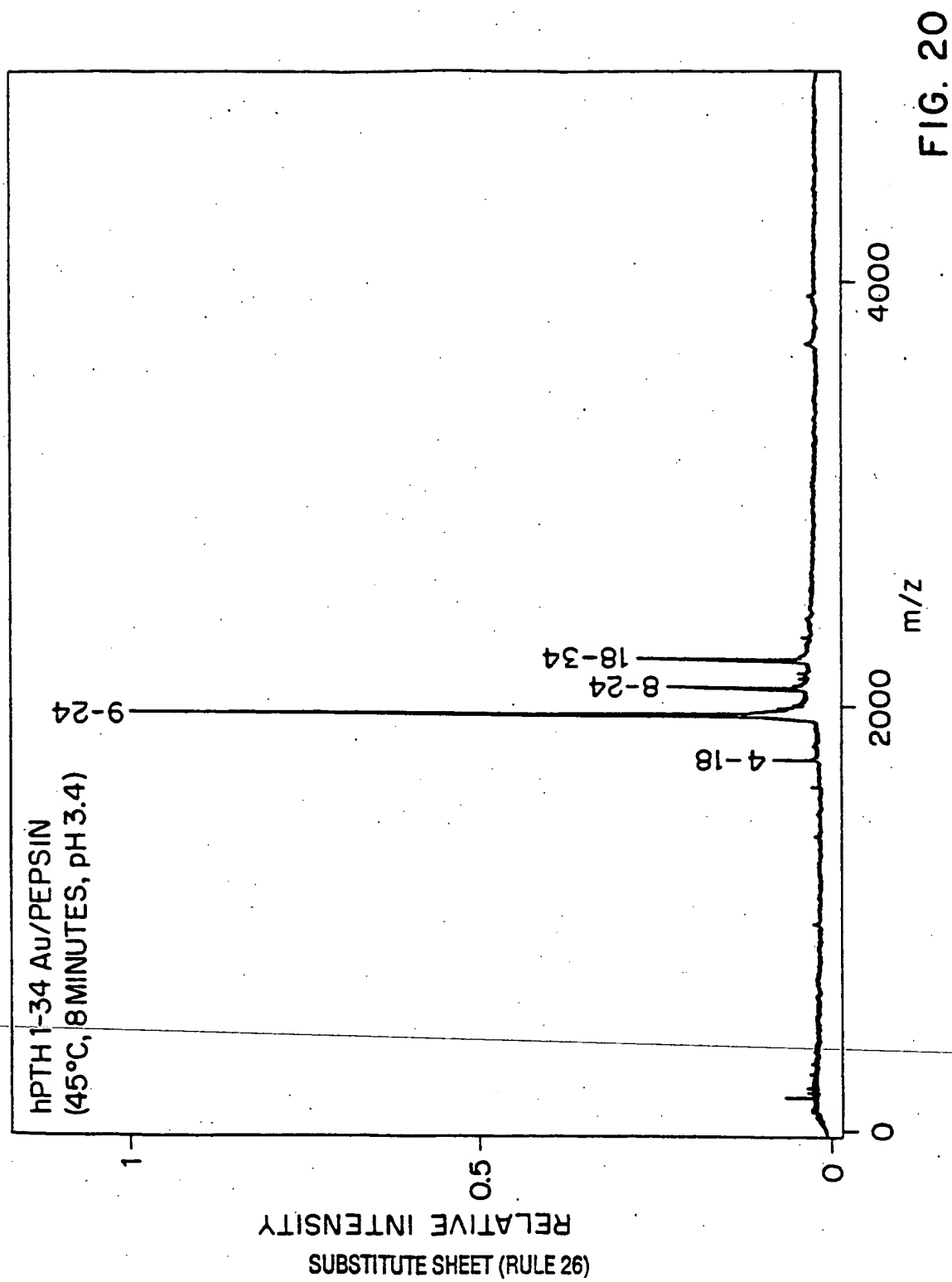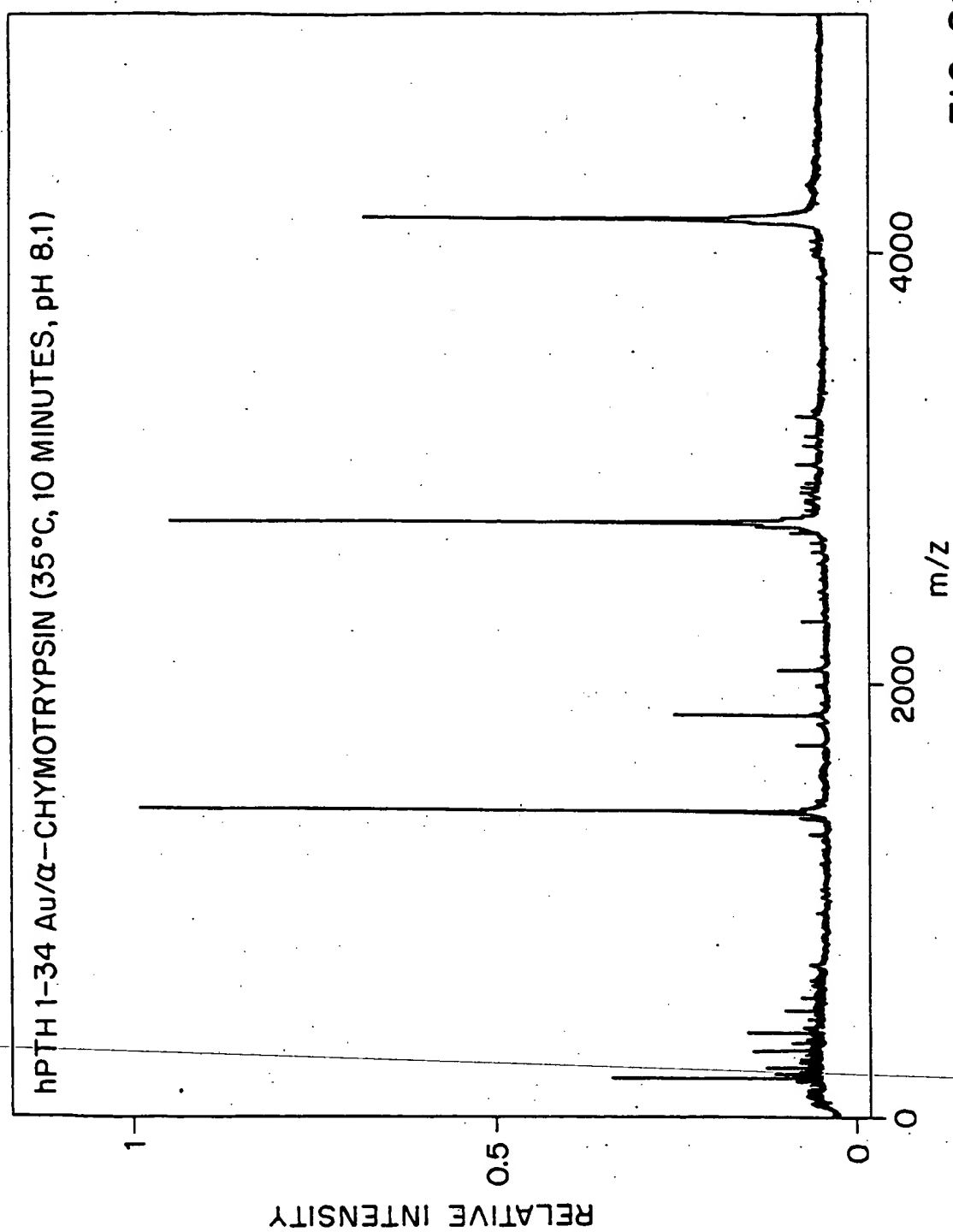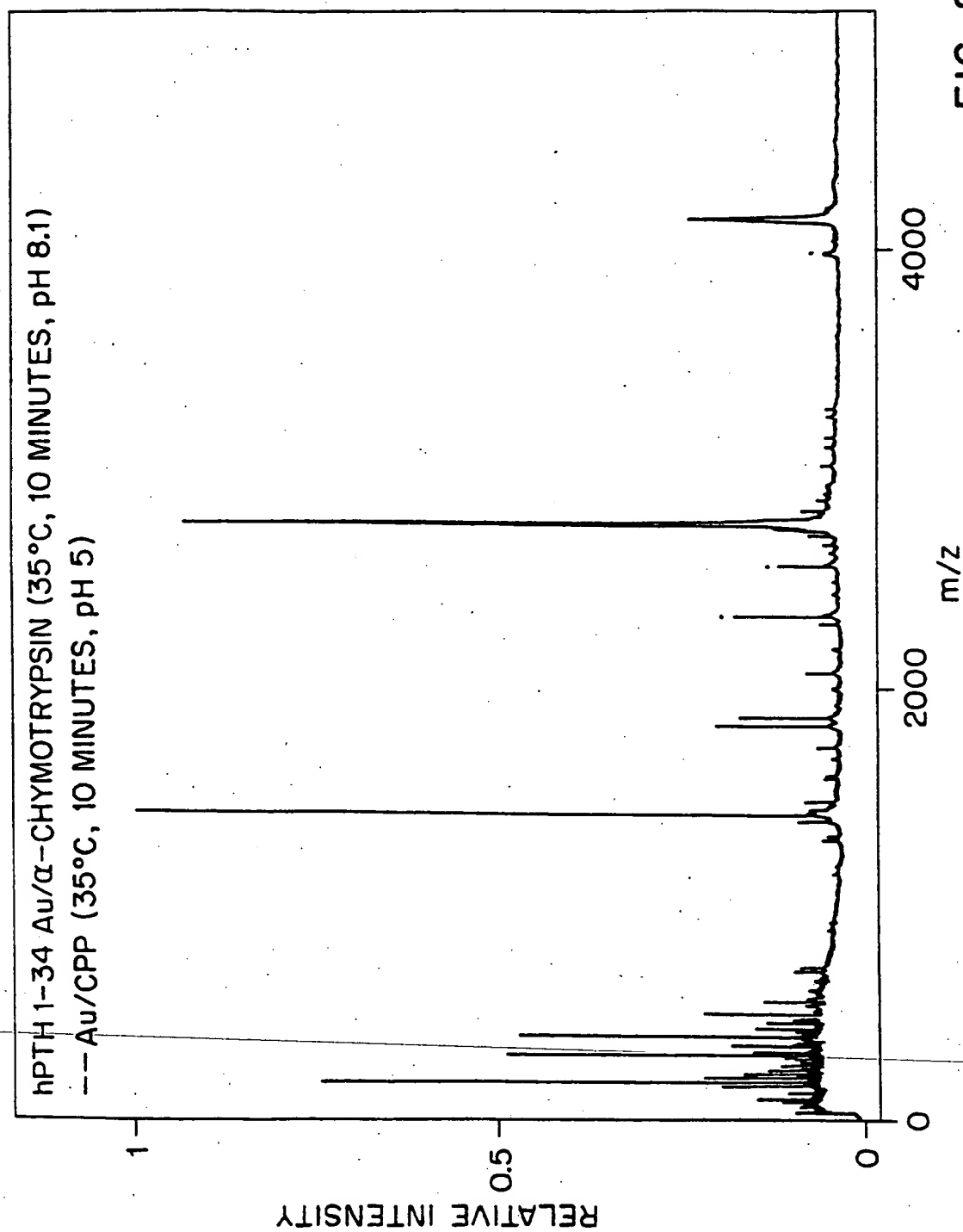
FIG. 19

FIG. 20

hPTH 1-34 Au/α-CHYMOTRYPSIN (35°C, 10 MINUTES, pH 8.1)

FIG. 21

FIG. 22

hPTH 1-34 Au/α-CHYMOTRYPSIN (35°C, 10 MINUTES, pH 8.1)
---Au/CPP (35°C, 10 MINUTES, pH 5)

RELATIVE INTENSITY

m/z

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :C12N.11/00; C12Q 1/37, 1/68; H01J 17/26, 27/00, 49/00
US CL   :250/281, 288, 423P; 313/566; 435/6, 23, 24, 174

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   250/281, 288, 423P; 313/566; 435/6, 23, 24, 174

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, CHEMISTRY ABSTRACTS, DERWENT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/28418 A1 (BAYLOR COLLEGE OF MEDICINE) 08 December 1994, see pages 4, 10, 17, 18, 26, 30-32, 48.	1-41
A	US 4,988,879 A (ZARE et al.) 29 January 1991, see entire document.	1-41
A, P	DOGRUEL, et al. Rapid Tryptic Mapping Using Enzymatically Active Mass Spectrometer Probe Tips. Analytical Chemistry. 01 December 1995, Vol. 67, No. 23, pages 4343-4348.	1-41

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 JULY 1996	30 SEP 1996

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer RALPH GITOMER
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.
                          ☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single
inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional
examination fees must be paid.

Group I, claim(s) 1-9, drawn to a sample presentation apparatus and method of making it.
Group II, claim(s) 10-41, drawn to a mass spec and method of using it.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under
PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The apparatus
and method of Group I is independent and does not require any feature of the apparatus and method of Group II. 37
CFR 1.475(d).